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<p>(21) International Application Number: PCT/CA97/00163</p> <p>(22) International Filing Date: 7 March 1997 (07.03.97)</p> <p>(30) Priority Data:</p> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">08/613,009</td> <td style="width: 33%;">8 March 1996 (08.03.96)</td> <td style="width: 33%; text-align: right;">US</td> </tr> <tr> <td>08/778,570</td> <td>3 January 1997 (03.01.97)</td> <td style="text-align: right;">US</td> </tr> </table> <p>(60) Parent Application or Grant</p> <p>(63) Related by Continuation</p> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">US</td> <td style="width: 33%; text-align: right;">08/778,570 (CIP)</td> <td style="width: 33%;"></td> </tr> <tr> <td>Filed on</td> <td style="text-align: right;">3 January 1997 (03.01.97)</td> <td></td> </tr> </table> <p>(71) Applicant (for all designated States except US): CONNAUGHT LABORATORIES LIMITED [CA/CA]; 1755 Steeles Avenue West, North York, Ontario M2R 3T4 (CA).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): MYERS, Lisa, E. [CA/CA]; 187 Elizabeth Street, Guelph, Ontario N1E 2X5 (CA). SCHRYVERS, Anthony, B. [CA/CA]; 39 Edforth Road, N.W., Calgary, Alberta T3A 3V8 (CA). HARKNESS, Robin, E. [CA/CA]; Apartment #1706, 640 Sheppard Avenue East, Willowdale, Ontario M2K 1B8 (CA). LOOSMORE, Sheena, M. [CA/CA]; 70 Crawford</p>	08/613,009	8 March 1996 (08.03.96)	US	08/778,570	3 January 1997 (03.01.97)	US	US	08/778,570 (CIP)		Filed on	3 January 1997 (03.01.97)		<p>Rose Drive, Aurora, Ontario L4G 4R4 (CA). DU, Run-Pan [CA/CA]; 299 Chelwood Drive, Thornhill, Ontario L4J 7Y8 (CA). YANG, Yan-Ping [CA/CA]; Apartment 1709, 120 Torresdale Avenue, Willowdale, Ontario M2R 3N7 (CA). KLEIN, Michel, H. [CA/CA]; 16 Munro Boulevard, Willowdale, Ontario M2P 1B9 (CA).</p> <p>(74) Agent: STEWART, Michael, I.; Sim & McBurney, 6th floor, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU. ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published</p> <p><i>With international search report.</i></p> <p><i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
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<p>(54) Title: TRANSFERRIN RECEPTOR GENES OF MORAXELLA</p> <p>(57) Abstract</p> <p>Purified and isolated nucleic acid molecules are provided which encode transferrin receptor proteins of <i>Moraxella</i>, such as <i>M. catarrhalis</i> or a fragment or an analog of the transferrin receptor protein. The nucleic acid sequence may be used to produce recombinant transferrin receptor proteins Tbp1 and Tbp2 of the strain of <i>Moraxella</i> free of other proteins of the <i>Moraxella</i> strain for purposes of diagnostics and medical treatment. Furthermore, the nucleic acid molecule may be used in the diagnosis of infection.</p>													

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TITLE OF INVENTION
TRANSFERRIN RECEPTOR GENES OF MORAXELLA

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FIELD OF INVENTION

The present invention relates to the molecular cloning of genes encoding transferrin receptor (TfR) proteins and, in particular, to the cloning of transferrin receptor genes from *Moraxella* (*Branhamella*) *catarrhalis*.

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REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of copending United States Patent Application No. 08/778,570 filed January 3, 1997, which itself is a continuation-in-part of United States Patent Application No. 08/613,009 filed March 8, 1996.

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BACKGROUND OF THE INVENTION

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Moraxella (*Branhamella*) *catarrhalis* bacteria are Gram-negative diplococcal pathogens which are carried asymptotically in the healthy human respiratory tract.

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In recent years, *M. catarrhalis* has been recognized as an important causative agent of otitis media. In addition, *M. catarrhalis* has been associated with sinusitis, conjunctivitis, and urogenital infections, as well as with a number of inflammatory diseases of the lower respiratory tract in children and adults, including pneumonia, chronic bronchitis, tracheitis, and emphysema (refs. 1 to 8). (Throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference

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into the present disclosure). Occasionally, *M. catarrhalis* invades to cause septicaemia, arthritis, endocarditis, and meningitis (refs. 9 to 13).

Otitis media is one of the most common illnesses of early childhood and approximately 80% of all children suffer at least one middle ear infection before the age of three (ref. 14). Chronic otitis media has been associated with auditory and speech impairment in children, and in some cases, has been associated with learning disabilities. Conventional treatments for otitis media include antibiotic administration and surgical procedures, including tonsillectomies, adenoidectomies, and tympanocentesis. In the United States, treatment costs for otitis media are estimated to be between one and two billion dollars per year.

In otitis media cases, *M. catarrhalis* commonly is co-isolated from middle ear fluid along with *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae*, which are believed to be responsible for 50% and 30% of otitis media infections, respectively. *M. catarrhalis* is believed to be responsible for approximately 20% of otitis media infections (ref. 15).

Epidemiological reports indicate that the number of cases of otitis media attributable to *M. catarrhalis* is increasing, along with the number of antibiotic-resistant isolates of *M. catarrhalis*. Thus, prior to 1970, no β -lactamase-producing *M. catarrhalis* isolates had been reported, but since the mid-seventies, an increasing number of β -lactamase-expressing isolates have been detected. Recent surveys suggest that 75% of clinical isolates produce β -lactamase (ref. 16, 26).

Iron is an essential nutrient for the growth of many bacteria. Several bacterial species, including *M. catarrhalis*, obtain iron from the host by using transferrin receptor proteins to capture transferrin. A number of bacteria including *Neisseria meningitidis*

(ref. 17), *N. gonorrhoeae* (ref. 18), *Haemophilus influenzae* (ref. 19), as well as *M. catarrhalis* (ref. 20), produce outer membrane proteins which specifically bind human transferrin. The expression of these proteins is regulated by the amount of iron in the environment.

The two transferrin receptor proteins of *M. catarrhalis*, designated transferrin binding protein 1 (Tbp1) and transferrin binding protein 2 (Tbp2), have molecular weights of 115 kDa (Tbp1) and approximately 80 to 90 kDa (Tbp2). Unlike the transferrin receptor proteins of other bacteria which have an affinity for apotransferrin, the *M. catarrhalis* Tbp2 receptors have a preferred affinity for iron-saturated (i.e., ferri-) transferrin (ref. 21).

M. catarrhalis infection may lead to serious disease. It would be advantageous to provide a recombinant source of transferrin binding proteins as antigens in immunogenic preparations including vaccines, carriers for other antigens and immunogens and the generation of diagnostic reagents. The genes encoding transferrin binding proteins and fragments thereof are particularly desirable and useful in the specific identification and diagnosis of *Moraxella* and for immunization against disease caused by *M. catarrhalis* and for the generation of diagnostic reagents.

SUMMARY OF THE INVENTION

The present invention is directed towards the provision of purified and isolated nucleic acid molecules encoding a transferrin receptor of a strain of *Moraxella* or a fragment or an analog of the transferrin receptor protein. The nucleic acid molecules provided herein are useful for the specific detection of strains of *Moraxella* and for diagnosis of infection by *Moraxella*. The purified and isolated nucleic acid

molecules provided herein, such as DNA, are also useful for expressing the *tbp* genes by recombinant DNA means for providing, in an economical manner, purified and isolated transferrin receptor proteins as well as subunits, fragments or analogs thereof. The transferrin receptor, subunits or fragments thereof or analogs thereof, as well as nucleic acid molecules encoding the same and vectors containing such nucleic acid molecules, are useful in immunogenic compositions for vaccinating against diseases caused by *Moraxella*, the diagnosis of infection by *Moraxella* and as tools for the generation of immunological reagents. Monoclonal antibodies or mono-specific antisera (antibodies) raised against the transferrin receptor protein, produced in accordance with aspects of the present invention, are useful for the diagnosis of infection by *Moraxella*, the specific detection of *Moraxella* (in, for example, *in vitro* and *in vivo* assays) and for the treatment of diseases caused by *Moraxella*.

In accordance with one aspect of the present invention, there is provided a purified and isolated nucleic acid molecule encoding a transferrin receptor protein of a strain of *Moraxella*, more particularly, a strain of *M. catarrhalis*, specifically *M. catarrhalis* strain 4223, Q8 or R1, or a fragment or an analog of the transferrin receptor protein.

In one preferred embodiment of the invention, the nucleic acid molecule may encode only the Tbp1 protein of the *Moraxella* strain or only the Tbp2 protein of the *Moraxella* strain. In another preferred embodiment of the invention, the nucleic acid may encode a fragment of the transferrin receptor protein of a strain of *Moraxella* having an amino acid sequence which is conserved.

In another aspect of the present invention, there is provided a purified and isolated nucleic acid

molecule having a DNA sequence selected from the group consisting of (a) a DNA sequence as set out in Figure 5, 6, 10, 11 or 27 (SEQ ID Nos: 1, 2, 3, 4, 5, 6, 7, 8, 45 or 46) or the complementary DNA sequence thereto; (b) a DNA sequence encoding an amino acid sequence as set out in Figure 5, 6, 10, 11 or 27 (SEQ ID Nos: 9, 10, 11, 12, 13, 14, 15, 16 or 47) or the complementary DNA sequence thereto; and (c) a DNA sequence which hybridizes under stringent conditions to any one of the DNA sequences defined in (a) or (b). The DNA sequence defined in (c) preferably has at least about 90% sequence identity with any one of the DNA sequences defined in (a) and (b). The DNA sequence defined in (c) may be that encoding the equivalent transferrin receptor protein from another strain of *Moraxella*.

In an additional aspect, the present invention includes a vector adapted for transformation of a host, comprising a nucleic acid molecule as provided herein and may have the characteristics of a nucleotide sequence contained within vectors LEM3-24, pLEM3, pLEM25, pLEM23, SLRD-A, DS-1698-1-1, DS-1754-1, pSLRD2, pSLRD3, pSLRD4 and pSLRD5.

The vector may be adapted for expression of the encoded transferrin receptor, fragments or analogs thereof, in a heterologous or homologous host, in either a lipidated or non-lipidated form. Accordingly, a further aspect of the present invention provides an expression vector adapted for transformation of a host comprising a nucleic acid molecule as provided herein and expression means operatively coupled to the nucleic acid molecule for expression by the host of the transferrin receptor protein or the fragment or analog of the transferrin receptor protein. In specific embodiments of this aspect of the invention, the nucleic acid molecule may encode substantially all the transferrin receptor protein, only the Tbp1 protein,

only the Tbp2 protein of the *Moraxella* strain or fragments of the Tbp1 or Tbp2 proteins. The expression means may include a promoter and a nucleic acid portion encoding a leader sequence for secretion from the host of the transferrin receptor protein or the fragment or the analog of the transferrin receptor protein. The expression means also may include a nucleic acid portion encoding a lipidation signal for expression from the host of a lipidated form of the transferrin receptor protein or the fragment or the analog of the transferrin receptor protein. The host may be selected from, for example, *Escherichia coli*, *Bordetella*, *Bacillus*, *Haemophilus*, *Moraxella*, fungi, yeast or baculovirus and Semliki Forest virus expression systems may be used. In a particular embodiment, the plasmid adapted for expression of Tbp1 is pLEM29 and that for expression of Tbp2 is pLEM33. Further vectors include pLEM-37, SLRD35-A and SLRD-35-B.

In an additional aspect of the invention, there is provided a transformed host containing an expression vector as provided herein. The invention further includes a recombinant transferrin receptor protein or fragment or analog thereof of a strain of *Moraxella* producible by the transformed host.

Such recombinant transferrin receptor protein may be provided in substantially pure form according to a further aspect of the invention, which provides a method of forming a substantially pure recombinant transferrin receptor protein, which comprises growing the transformed host provided herein to express a transferrin receptor protein as inclusion bodies, purifying the inclusion bodies free from cellular material and soluble proteins, solubilizing transferrin receptor protein from the purified inclusion bodies, and purifying the transferrin receptor protein free from other solubilized materials. The substantially pure

recombinant transferrin receptor protein may comprise Tbp1 alone, Tbp2 alone or a mixture thereof. The recombinant protein is generally at least about 70% pure, preferably at least about 90% pure.

5 Further aspects of the present invention, therefore, provide recombinantly-produced Tbp1 protein of a strain of *Moraxella* devoid of the Tbp2 protein of the *Moraxella* strain and any other protein of the *Moraxella* strain and recombinantly-produced Tbp2 protein
10 of a strain of *Moraxella* devoid of the Tbp1 protein of the *Moraxella* strain and any other protein of the *Moraxella* strain. The *Moraxella* strain may be *M. catarrhalis* 4223 strain, *M. catarrhalis* Q8 strain or *M. catarrhalis* R1 strain.

15 In accordance with another aspect of the invention, an immunogenic composition is provided which comprises at least one active component selected from at least one nucleic acid molecule as provided herein and at least one recombinant protein as provided herein, and a
20 pharmaceutically acceptable carrier therefor or vector therefor. The at least one active component produces an immune response when administered to a host.

The immunogenic compositions provided herein may be formulated as vaccines for in vivo administration to a
25 host. For such purpose, the compositions may be formulated as a microparticle, capsule, ISCOM or liposome preparation. The immunogenic composition may be provided in combination with a targeting molecule for delivery to specific cells of the immune system or to
30 mucosal surfaces. The immunogenic compositions of the invention (including vaccines) may further comprise at least one other immunogenic or immunostimulating material and the immunostimulating material may be at least one adjuvant or at least one cytokine. Suitable
35 adjuvants for use in the present invention include (but are not limited to) aluminum phosphate, aluminum

hydroxide, QS21, Quil A, derivatives and components thereof, ISCOM matrix, calcium phosphate, calcium hydroxide, zinc hydroxide, a glycolipid analog, an octadecyl ester of an amino acid, a muramyl dipeptide polyphosphazene, ISCOPREP, DC-chol, DDBA and a lipoprotein. Advantageous combinations of adjuvants are described in copending United States Patent Applications Nos. 08/261,194 filed June 16, 1994 and 08/483,856, filed June 7, 1995, assigned to the assignee hereof and the disclosures of which are incorporated herein by reference thereto (WO 95/34308).

In accordance with another aspect of the invention, there is provided a method for generating an immune response in a host, comprising the step of administering to a susceptible host, such as a human, an effective amount of the immunogenic composition provided herein. The immune response may be a humoral or a cell-mediated immune response and may provide protection against disease caused by *Moraxella*. Hosts in which protection against disease may be conferred include primates, including humans.

In a further aspect, there is provided a live vector for delivery of transferrin receptor to a host, comprising a vector containing the nucleic acid molecule as described above. The vector may be selected from *Salmonella*, BCG, adenovirus, poxvirus, vaccinia and poliovirus.

The nucleic acid molecules provided herein are useful in diagnostic applications. Accordingly, in a further aspect of the invention, there is provided a method of determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising the steps of:

(a) contacting the sample with a nucleic acid molecule as provided herein to produce duplexes comprising the nucleic acid molecule and any nucleic

acid molecule encoding the transferrin receptor protein of a strain of *Moraxella* present in the sample and specifically hybridizable therewith; and

(b) determining the production of the duplexes.

5 In addition, the present invention provides a diagnostic kit for determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising:

(a) a nucleic acid molecule as provided herein;

10 (b) means for contacting the nucleic acid molecule with the sample to produce duplexes comprising the nucleic acid molecule and any such nucleic acid present in the sample and hybridizable with the nucleic acid molecule; and

15 (c) means for determining production of the duplexes.

The invention further includes the use of the nucleic acid molecules and proteins provided herein as medicines. The invention additionally includes the use
20 of the nucleic acid molecules and proteins provided herein in the manufacture of medicaments for protection against infection by strains of *Moraxella*.

Advantages of the present invention include:

- an isolated and purified nucleic acid
25 molecule encoding a transferrin receptor protein of a strain of *Moraxella* or a fragment or an analog of the transferrin receptor protein;

- recombinantly-produced transferrin receptor proteins, including Tbp1 and Tbp2, free from each other
30 and other *Moraxella* proteins; and

- diagnostic kits and immunological reagents for specific identification of *Moraxella*.

BRIEF DESCRIPTION OF DRAWINGS

35 The present invention will be further understood from the following description with reference to the

drawings, in which:

Figure 1 shows the amino acid sequences (SEQ ID Nos: 17 and 18) of a conserved portion of Tbp1 proteins used for synthesis of degenerate primers used for PCR amplification of a portion of the *M. catarrhalis* 4223 *tbpA* gene;

Figure 2 shows a restriction map of clone LEM3-24 containing the *tbpA* and *tbpB* genes from *M. catarrhalis* isolate 4223;

Figure 3 shows a restriction map of the *tbpA* gene for *M. catarrhalis* 4223;

Figure 4 shows a restriction map of the *tbpB* gene for *M. catarrhalis* 4223;

Figure 5 shows the nucleotide sequence of the *tbpA* gene (SEQ ID No: 1 - entire sequence and SEQ ID No: 2 - coding sequence) and the deduced amino acid sequence of the Tbp1 protein from *M. catarrhalis* 4223 (SEQ ID No: 9 - full length and SEQ ID No: 10 - mature protein). The leader sequence (SEQ ID No: 19) is shown by underlining;

Figure 6 shows the nucleotide sequence of the *tbpB* gene (SEQ ID No: 3 - entire sequence and SEQ ID No: 4 - coding sequence) and the deduced amino acid sequence of the Tbp2 protein from *M. catarrhalis* 4223 (SEQ ID Nos: 11 - full length and SEQ ID No: 12 - mature protein). The leader sequence (SEQ ID No: 20) is shown by underlining;

Figure 7 shows a restriction map of clone SLRD-A containing the *tbpA* and *tbpB* genes from *M. catarrhalis* Q8;

Figure 8 shows a restriction map of the *tbpA* gene from *M. catarrhalis* Q8;

Figure 9 shows a restriction map of the *tbpB* gene from *M. catarrhalis* Q8;

Figure 10 shows the nucleotide sequence of the *tbpA* gene (SEQ. ID No: 5 - entire sequence and SEQ ID No: 6 - coding sequence) and the deduced amino acid sequence of

the Tbp1 protein from *M. catarrhalis* Q8 (SEQ ID No: 13 - full length and SEQ ID No: 14 - mature protein);

Figure 11 shows the nucleotide sequence of the *tbpB* gene (SEQ. ID No: 7 - entire sequence and SEQ ID No: 8 - coding sequence) and the deduced amino acid sequence of the Tbp2 protein from *M. catarrhalis* Q8 (SEQ ID No: 15 - full length and SEQ ID No: 16 - mature protein);

Figure 12 shows a comparison of the amino acid sequences of Tbp1 from *M. catarrhalis* strain 4223 (SEQ ID No: 9) and Q8 (SEQ ID No: 13), *H. influenzae* strain Eagan (SEQ ID No: 21), *N. meningitidis* strains B16B6 (SEQ ID No: 22) and M982 (SEQ ID No: 23), and *N. gonorrhoeae* strain FA19 (SEQ ID No: 24). Dots indicate identical residues and dashes have been inserted for maximum alignment;

Figure 13 shows a comparison of the amino acid sequences of Tbp2 from *M. catarrhalis* isolate 4223 (SEQ ID No: 11) and Q8 (SEQ ID No: 15), *H. influenzae* strain Eagan (SEQ ID No: 25), *N. meningitidis* strains B16B6 (SEQ ID No: 26) and M918 (SEQ ID No: 27), and *N. gonorrhoeae* strain FA19 (SEQ ID No: 28). Dots indicate identical residues and dashes have been inserted for maximum alignment;

Figure 14 shows the construction of plasmid pLEM29 for expression of recombinant Tbp1 protein from *E. coli*;

Figure 15 shows an SDS-PAGE analysis of the expression of Tbp1 protein by *E. coli* cells transformed with plasmid pLEM29;

Figure 16 shows a flow chart for purification of recombinant Tbp1 protein;

Figure 17 shows an SDS-PAGE analysis of purified recombinant Tbp1 protein;

Figure 18 shows the construction of plasmid pLEM33 and pLEM37 for expression of TbpA gene from *M. catarrhalis* 4223 in *E. coli* without and with a leader sequence respectively;

Figure 19 shows an SDS-PAGE analysis of the expression of rTbp2 protein by *E. coli* cells transformed with plasmid pLEM37;

5 Figure 20 shows the construction of plasmid sLRD35B for expression of the *tbpB* gene from *M. catarrhalis* Q8 in *E. coli* without a leader sequence, and the construction of plasmid SLRD35A for expression of the *tbpB* gene from *M. catarrhalis* Q8 in *E. coli* with a leader sequence. Restriction site B = BamHI; Bg = Bgl
10 II; H = Hind III; R = EcoRI;

Figure 21 shows SDS PAGE analysis of the expression of rTbp2 protein in *E. coli* cells, transformed with plasmids SLRD35A and SLRD35B;

15 Figure 22 shows a flow chart for purification of recombinant Tbp2 protein from *E. coli*;

Figure 23, which includes Panels A and B, shows an SDS-PAGE analysis of the purification of recombinant Tbp2 protein from *M. catarrhalis* strains 4223 (Panel A) and Q8 (Panel B) from expression in *E. coli*;

20 Figure 24 shows the binding of Tbp2 to human transferrin;

Figure 25, which includes Panels A, B and C, shows the antigenic conservation of Tbp2 protein amongst strains of *M. catarrhalis*;

25 Figure 26 shows a restriction map of the *tbpB* gene for *M. catarrhalis* R1;

Figure 27 shows the nucleotide sequence of the *tbpB* gene (SEQ ID No: 45 - entire sequence and SEQ ID No: 46 - coding sequence) and the deduced amino acid
30 sequence of the Tbp2 protein of *M. catarrhalis* R1 (SEQ ID No: 47); and

Figure 28 shows a comparison of the amino acid sequences of Tbp2 for *M. catarrhalis* 4223 (SEQ ID No: 21), Q8 (SEQ ID No: 15) and R1 (SEQ ID No: 47). Dots
35 indicate identical residues and dashes have been inserted for maximum alignment. The asterisks indicate

stop codons.

GENERAL DESCRIPTION OF THE INVENTION

Any *Moraxella* strain may be conveniently used to provide the purified and isolated nucleic acid, which may be in the form of DNA molecules, comprising at least a portion of the nucleic acid coding for a transferrin receptor as typified by embodiments of the present invention. Such strains are generally available from clinical sources and from bacterial culture collections, such as the American Type Culture Collection.

In this application, the terms "transferrin receptor" (TfR) and "transferrin binding proteins" (Tbp) are used to define a family of Tbp1 and/or Tbp2 proteins which includes those having variations in their amino acid sequences including those naturally occurring in various strains of, for example, *Moraxella*. The purified and isolated DNA molecules comprising at least a portion coding for transferrin receptor of the present invention also include those encoding functional analogs of transferrin receptor proteins Tbp1 and Tbp2 of *Moraxella*. In this application, a first protein is a "functional analog" of a second protein if the first protein is immunologically related to and/or has the same function as the second protein. The functional analog may be, for example, a fragment of the protein, or a substitution, addition or deletion mutant thereof.

Chromosomal DNA from *M. catarrhalis* 4223 was digested with *Sau3A* in order to generate fragments within a 15 to 23 kb size range, and cloned into the *Bam*HI site of the lambda vector EMBL3. The library was screened with anti-Tbp1 guinea pig antisera, and a positive clone LEM3-24, containing an insert approximately 13.2 kb in size was selected for further analysis. Lysate from *E. coli* LE392 infected with LEM3-24 was found to contain a protein approximately 115 kDa

in size, which reacted on Western blots with anti-Tbpl antisera. A second protein, approximately 80 kDa in size, reacted with the anti-Tbp2 guinea pig antisera on Western blots.

5 In order to localize the *tbpA* gene on the 13.2 kb insert of LEM3-24, degenerate PCR primers were used to amplify a small region of the putative *tbpA* gene of *M. catarrhalis* 4223. The sequences of the degenerate oligonucleotide primers were based upon conserved amino
10 acid sequences within the Tbpl proteins of several *Neisseria* and *Haemophilus* species and are shown in Figure 1 (SEQ ID Nos: 17 and 18). A 300 base-pair amplified product was generated and its location within the 4223 *tbpA* gene is indicated by bold letters in
15 Figure 5 (SEQ ID No: 29). The amplified product was subcloned into the vector pCRII, labelled, and used to probe a Southern blot containing restriction-endonuclease digested clone LEM3-24 DNA. The probe hybridized to a 3.8 kb *HindIII-HindIII*, a 2.0 kb *AvrII-AvrII*, and 4.2 kb *SalI-SphI* fragments (Figure 2).

20 The 3.8 kb *HindIII-HindIII* fragment was subcloned into pACYC177, and sequenced. A large open reading frame was identified, and subsequently found to contain approximately 2 kb of the putative *tbpA* gene. The
25 remaining 1 kb of the *tbpA* gene was obtained by subcloning an adjacent downstream *HindIII-HindIII* fragment into vector pACYC177. The nucleotide sequence of the *tbpA* gene from *M. catarrhalis* 4223 (SEQ ID Nos: 1 and 2), and the deduced amino acid sequence (SEQ ID
30 No: 9 - full length; SEQ ID No: 10 mature protein) are shown in Figure 5.

35 Chromosomal DNA from *M. catarrhalis* strain Q8 was digested with *Sau3A I* and 15-23 kb fragments were ligated with *BamHI* arms of EMBL3. A high titre library was generated in *E. coli* LE392 cells and was screened using oligonucleotide probes based on the 4223 *tbpA*

sequence. Phage DNA was prepared and restriction enzyme analysis revealed that inserts of about 13-15 kb had been cloned. Phage clone SLRD-A was used to subclone fragments for sequence analysis. A cloning vector (pSKMA) was generated to facilitate cloning of the fragments and plasmids pSLRD1, pSLRD2, pSLRD3, pSLRD4 and pSLRD5 were generated which contain all of *tbpA* and most of *tbpB*. The nucleotide (SEQ ID Nos: 5 and 6) and deduced amino acid sequence (SEQ ID No: 13 - full length, SEQ ID No: 14 - mature protein) of the *tbpA* gene from strain Q8 are shown in Figure 10.

The deduced amino acid sequences for the Tbp1 protein encoded by the *tbpA* genes were found to share some homology with the amino acid sequences encoded by genes from a number of *Neisseria* and *Haemophilus* species (Figure 12; SEQ ID Nos: 21, 22, 23 and 24).

Prior to the present discovery, *tbpA* genes identified in species of *Neisseria*, *Haemophilus*, and *Actinobacillus* have been found to be preceded by a *tbpB* gene with several conserved regions. The two genes typically are separated by a short intergenic sequence. However, a *tbpB* gene was not found upstream of the *tbpA* gene in *M. catarrhalis* 4223. In order to localize the *tbpB* gene within the 13.2 kb insert of clone LEM3-24, a denenerate oligonucleotide probe was synthesized based upon an amino acid sequence EGGFYGP (SEQ ID No: 30), conserved among Tbp2 proteins of several species. The oligonucleotide was labelled and used to probe a Southern blot containing different restriction endonuclease fragments of clone LEM3-24. The probe hybridized to a 5.5 kb *NheI*-*SalI* fragment, which subsequently was subcloned into pBR328, and sequenced. The fragment contained most of the putative *tbpB* gene, with the exception of the promoter region. The clone LEM3-24 was sequenced to obtain the remaining upstream sequence. The *tbpB* gene was located approximately 3 kb

downstream from the end of the *tbpA* gene, in contrast to the genetic organization of the *tbpA* and *tbpB* genes in *Haemophilus* and *Neisseria*. The nucleotide sequence (SEQ ID Nos: 3 and 4) of the *tbpB* gene from *M. catarrhalis* 4223 and the deduced amino acid sequence (SEQ ID Nos: 11, 12) are shown in Figure 6. The *tbpB* gene from *M. catarrhalis* Q8 was also cloned and sequenced. The nucleotide sequence (SEQ ID Nos: 7 and 8) and the deduced amino acid sequence (SEQ ID Nos: 15 and 16) are shown in Figure 11. The *tbpB* gene from *M. catarrhalis* R1 was also cloned and sequenced. The nucleotide sequence (SEQ ID Nos: 45 and 46) and the deduced amino acid sequence (SEQ ID No: 47) are shown in Figure 27. Regions of homology are evident between the *M. catarrhalis* Tbp2 amino acid sequences as shown in the comparative alignment of Figure 28 (SEQ ID Nos: 11, 15 and 47) and between the *M. catarrhalis* Tbp2 amino acid sequences and the Tbp2 sequences of a number of *Neisseria* and *Haemophilus* species, as shown in the comparative alignment in Figure 13 (SEQ ID Nos: 25, 26, 27, 28).

Cloned *tbpA* and *tbpB* genes were expressed in *E. coli* to produce recombinant Tbp1 and Tbp2 proteins free of other *Moraxella* proteins. These recombinant proteins were purified and used for immunization.

The antigenic conservation of Tbp2 protein amongst strains of *M. catarrhalis* was demonstrated by separation of the proteins in whole cell lysates of *M. catarrhalis* or strains of *E. coli* expressing recombinant Tbp2 proteins by SDS PAGE and antiserum immunoblotting with anti-4223 rTbp2 antiserum or anti-Q8 rTbp2 antiserum raised in guinea pigs. *M. catarrhalis* strains 3, 56, 135, 585, 4223, 5191, 8185 and ATCC 25240 were tested in this way and all showed specific reactivity with anti-4223 rTbp2 or anti-Q8 rTbp2 antibody (Figure 25).

In addition, the ability of anti-rTbp2 antibodies from one strain to recognize native or recombinant protein from the homologous or heterologous strain by ELISA is shown in Table 1 below.

5 Amino acid sequencing of the N-termini and cyanogen bromide fragments of transferrin receptor from *M. catarrhalis* 4223 was undertaken. Both N-termini of Tbp1 and Tbp2 were blocked. The putative signal sequences of Tbp1 and Tbp2 are indicated by underlining in Figures 5 and 6 (SEQ ID Nos: 19 and 20) respectively. The deduced amino acid sequences for the N-terminal region of Tbp2 suggests a lipoprotein structure.

10 Results shown in Tables 1 and 2 below illustrate the ability of anti-Tbp1 and anti-Tbp2 guinea pig antisera, produced by the immunization with Tbp1 or Tbp2, to lyse *M. catarrhalis*. The results show that the antisera produced by immunization with Tbp1 or Tbp2 protein isolated from *M. catarrhalis* isolate 4223 were bactericidal against a homologous non-clumping *M. catarrhalis* strain RH408 (a strain previously deposited in connection with United States Patent Application No. 08/328,589, assigned to the assignee hereof, (WO 96/12733) with the American Type Culture Collection, located at 1301 Parklawn Drive, Rockville, Maryland 20852, USA under the terms of the Budapest Treaty on December 13, 1994 under ATCC Deposit No. 55,637) derived from isolate 4223. In addition, antisera produced by immunization with Tbp1 protein isolated from *M. catarrhalis* 4223 were bactericidal against the heterologous non-clumping strain Q8 (a gift from Dr. M.G. Bergeron, Centre Hospitalier de l'Université Laval, St. Foy, Quebec). In addition, antiserum raised against recombinant Tbp2 (rTbp2) protein was bactericidal against the homologous strain of *M. catarrhalis*.

35 The ability of isolated and purified transferrin binding proteins to generate bactericidal antibodies is

in vivo evidence of utility of these proteins as vaccines to protect against disease caused by *Moraxella*.

Thus, in accordance with another aspect of the present invention, there is provided a vaccine against infection caused by *Moraxella* strains, comprising an immunogenically-effective amount of a transferrin binding protein from a strain of *Moraxella* and a physiologically-acceptable carrier therefor. Vaccine preparations may comprise antigenically or sequence divergent transferrin binding proteins.

The transferrin binding protein provided herein is useful as a diagnostic reagent, as an antigen for the generation of anti-transferrin protein binding antibodies, as an antigen for vaccination against the disease caused by species of *Moraxella* and for detecting infection by *Moraxella* and other such bacteria.

The transferrin binding protein provided herein may also be used as a carrier protein for haptens, polysaccharides or peptides to make conjugate vaccines against antigenic determinants unrelated to transferrin binding proteins. In additional embodiments of the present invention, therefore, the transferrin binding protein as provided herein may be used as a carrier molecule to prepare chimeric molecules and conjugate vaccines (including glycoconjugates) against pathogenic bacteria, including encapsulated bacteria. Thus, for example, glycoconjugates of the present invention may be used to confer protection against disease and infection caused by any bacteria having polysaccharide antigens including lipooligosaccharides (LOS) and PRP. Such bacterial pathogens may include, for example, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Escherichia coli*, *Neisseria meningitidis*, *Salmonella typhi*, *Streptococcus mutans*, *Cryptococcus neoformans*, *Klebsiella*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Particular antigens which can be conjugated

to transferrin binding protein and methods to achieve such conjugations are described in U.S. Patent Application No. 08/433,522 filed November 23, 1993 (WO 94/12641), assigned to the assignee hereof and the disclosure of which is hereby incorporated by reference thereto.

In another embodiment, the carrier function of transferrin binding protein may be used, for example, to induce an immune response against abnormal polysaccharides of tumour cells, or to produce anti-tumour antibodies that can be conjugated to chemotherapeutic or bioactive agents.

The invention extends to transferrin binding proteins from *Moraxella catarrhalis* for use as an active ingredient in a vaccine against disease caused by infection with *Moraxella*. The invention also extends to a pharmaceutical vaccinal composition containing transferrin binding proteins from *Moraxella catarrhalis* and optionally, a pharmaceutically acceptable carrier and/or diluent.

In a further aspect the invention provides the use of transferrin binding proteins for the preparation of a pharmaceutical vaccinal composition for immunization against disease caused by infection with *Moraxella*.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis, treatment of, for example, *Moraxella* infections and the generation of immunological and other diagnostic reagents. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from immunogenic transferrin receptor proteins, analogs and fragments thereof encoded by the nucleic acid molecules as well as the nucleic

acid molecules disclosed herein. The vaccine elicits an immune response which produces antibodies, including anti-transferrin receptor antibodies and antibodies that are opsonizing or bactericidal. Should the vaccinated
5 subject be challenged by *Moraxella*, the antibodies bind to the transferrin receptor and thereby prevent access of the bacteria to an iron source which is required for viability. Furthermore, opsonizing or bactericidal anti-transferrin receptor antibodies may also provide
10 protection by alternative mechanisms.

Immunogenic compositions, including vaccines, may be prepared as injectables, as liquid solutions or emulsions. The transferrin receptor proteins, analogs and fragments thereof and encoding nucleic acid
15 molecules may be mixed with pharmaceutically acceptable excipients which are compatible with the transferrin receptor proteins, fragments, analogs or nucleic acid molecules. Such excipients may include water, saline, dextrose, glycerol, ethanol, and combinations thereof.
20 The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants, to enhance the effectiveness of the vaccines. Immunogenic compositions and vaccines may be
25 administered parenterally, by injection subcutaneously, intradermally or intramuscularly. Alternatively, the immunogenic compositions provided according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces.
30 Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastic) routes. The immunogenic composition may be provided in combination with a targeting molecule for delivery to specific cells of the immune system or to
35 mucosal surfaces. Some such targeting molecules include vitamin B12 and fragments of bacterial toxins, as

described in WO 92/17167 (Biotech Australia Pty. Ltd.), and monoclonal antibodies, as described in U.S. Patent No. 5,194,254 (Barber et al). Alternatively, other modes of administration, including suppositories and oral formulations, may be desirable. For suppositories, binders and carriers may include, for example, polyalkalene glycols or triglycerides. Oral formulations may include normally employed incipients such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate. These compositions may take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 1 to 95% of the transferrin receptor proteins, fragments, analogs and/or nucleic acid molecules.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies, and, if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms of the transferrin receptor proteins, analogs and fragments thereof and/or nucleic acid molecules. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage of the vaccine may also depend on the route of administration and will vary according to the size of the host.

The nucleic acid molecules encoding the transferrin

receptor of *Moraxella* may be used directly for immunization by administration of the DNA directly, for example, by injection for genetic immunization or by constructing a live vector, such as *Salmonella*, BCG, adenovirus, poxvirus, vaccinia or poliovirus containing the nucleic acid molecules. A discussion of some live vectors that have been used to carry heterologous antigens to the immune system is contained in, for example, O'Hagan (ref 22). Processes for the direct injection of DNA into test subjects for genetic immunization are described in, for example, Ulmer et al. (ref. 23).

Immunogenicity can be significantly improved if the antigens are co-administered with adjuvants, commonly used as an 0.05 to 1.0 percent solution in phosphate - buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use

in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established and an HBsAg vaccine has been adjuvanted with alum. While the usefulness of alum is well established for some applications, it has limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response. The antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection by some vaccinal agents.

A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria and mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

To efficiently induce humoral immune responses (HIR) and cell-mediated immunity (CMI), immunogens are often emulsified in adjuvants. Many adjuvants are toxic, inducing granulomas, acute and chronic inflammations (Freund's complete adjuvant, FCA), cytotoxicity (saponins and pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (LPS and MDP). Although FCA is an excellent adjuvant and widely used in research, it is not licensed for use in human or veterinary vaccines because of its toxicity.

Desirable characteristics of ideal adjuvants include:

- (1) lack of toxicity;
- (2) ability to stimulate a long-lasting immune

response;

(3) simplicity of manufacture and stability in long-term storage;

(4) ability to elicit both CMI and HIR to antigens administered by various routes, if required;

(5) synergy with other adjuvants;

(6) capability of selectively interacting with populations of antigen presenting cells (APC);

(7) ability to specifically elicit appropriate T_H1 or T_H2 cell-specific immune responses; and

(8) ability to selectively increase appropriate antibody isotype levels (for example, IgA) against antigens.

U.S. Patent No. 4,855,283 granted to Lockhoff et al on August 8, 1989, which is incorporated herein by reference thereto, teaches glycolipid analogues including N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. Thus, Lockhoff et al. 1991 (ref. 24) reported that N-glycolipid analogs displaying structural similarities to the naturally-occurring glycolipids, such as glycopospholipids and glycoglycerolipids, are capable of eliciting strong immune responses in both herpes simplex virus vaccine and pseudorabies virus vaccine. Some glycolipids have been synthesized from long chain-alkylamines and fatty acids that are linked directly with the sugars through the anomeric carbon atom, to mimic the functions of the naturally occurring lipid residues.

U.S. Patent No. 4,258,029 granted to Moloney, assigned to the assignee hereof and incorporated herein by reference thereto, teaches that octadecyl tyrosine hydrochloride (OTH) functions as an adjuvant when complexed with tetanus toxoid and formalin inactivated type I, II and III poliomyelitis virus vaccine. Also,

Nixon-George et al. 1990, (ref. 25) reported that octadecyl esters of aromatic amino acids complexed with a recombinant hepatitis B surface antigen, enhanced the host immune responses against hepatitis B virus.

5 **2. Immunoassays**

 The transferrin receptor proteins, analogs and/or fragments thereof of the present invention are useful as immunogens, as antigens in immunoassays including enzyme-linked immunosorbent assays (ELISA), RIAs and
10 other non-enzyme linked antibody binding assays or procedures known in the art for the detection of anti-*Moraxella*, transferrin receptor protein antibodies. In ELISA assays, the transferrin receptor protein, analogs and/or fragments corresponding to portions of TfR
15 protein, are immobilized onto a selected surface, for example, a surface capable of binding proteins or peptides such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed transferrin receptor, analogs and/or fragments, a non-
20 specific protein such as a solution of bovine serum albumin (BSA) or casein that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the
25 immobilizing surface and thus reduces the background caused by non-specific bindings of antisera onto the surface.

 The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be
30 tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with diluents, such as BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to
35 incubate for from about 2 to 4 hours, at temperatures such as of the order of about 25° to 37°C. Following

incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution such as PBS/Tween or a borate buffer.

5 Following formation of specific immunocomplexes between the test sample and the bound transferrin receptor protein, analogs and/or fragments and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined by subjecting
10 the immunocomplex to a second antibody having specificity for the first antibody. If the test sample is of human origin, the second antibody is an antibody having specificity for human immunoglobulins and in general IgG. To provide detecting means, the second
15 antibody may have an associated activity such as an enzymatic activity that will generate, for example, a color development upon incubating with an appropriate chromogenic substrate. Quantification may then be achieved by measuring the degree of color generation using, for
20 example, a spectrophotometer.

3. Use of Sequences as Hybridization Probes

 The nucleotide sequences of the present invention, comprising the sequence of the transferrin receptor gene, now allow for the identification and cloning of
25 the transferrin receptor genes from any species of *Moraxella*.

 The nucleotide sequences comprising the sequence of the transferrin receptor genes of the present invention are useful for their ability to selectively form duplex
30 molecules with complementary stretches of other TfR genes. Depending on the application, a variety of hybridization conditions may be employed to achieve varying degrees of selectivity of the probe toward the other TfR genes. For a high degree of selectivity,
35 relatively stringent conditions are used to form the duplexes, such as low salt and/or high temperature

conditions, such as provided by 0.02 M to 0.15 M NaCl at temperatures of between about 50°C to 70°C. For some applications, less stringent hybridization conditions are required such as 0.15 M to 0.9 M salt, at temperatures ranging from between about 20°C to 55°C. Hybridization conditions can also be rendered more stringent by the addition of increasing amounts of formamide, to destabilize the hybrid duplex. Thus, particular hybridization conditions can be readily manipulated, and will generally be a method of choice depending on the desired results. In general, convenient hybridization temperatures in the presence of 50% formamide are: 42°C for a probe which is 95 to 100% homologous to the target fragment, 37°C for 90 to 95% homology and 32°C for 85 to 90% homology.

In a clinical diagnostic embodiment, the nucleic acid sequences of the TfR genes of the present invention may be used in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin and digoxigenin-labelling, which are capable of providing a detectable signal. In some diagnostic embodiments, an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of a radioactive tag may be used. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with samples containing TfR gene sequences.

The nucleic acid sequences of TfR genes of the present invention are useful as hybridization probes in solution hybridizations and in embodiments employing solid-phase procedures. In embodiments involving solid-

phase procedures, the test DNA (or RNA) from samples, such as clinical samples, including exudates, body fluids (e. g., serum, amniotic fluid, middle ear effusion, sputum, bronchoalveolar lavage fluid) or even tissues, is adsorbed or otherwise affixed to a selected matrix or surface. The fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes comprising the nucleic acid sequences of the TfR genes or fragments thereof of the present invention under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required depending on, for example, the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe etc. Following washing of the hybridization surface so as to remove non-specifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label. It is preferred to select nucleic acid sequence portions which are conserved among species of *Moraxella*. The selected probe may be at least 18bp and may be in the range of about 30 to 90 bp.

4. Expression of the Transferrin Receptor Genes

Plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell may be used for the expression of the transferrin receptor genes in expression systems. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* may be transformed using pBR322 which contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage, must also contain, or be modified to contain, promoters which can be used by the host cell for

expression of its own proteins.

In addition, phage vectors containing replicon and control sequences that are compatible with the host can be used as a transforming vector in connection with these hosts. For example, the phage in lambda GEMTM-11 may be utilized in making recombinant phage vectors which can be used to transform host cells, such as *E. coli* LE392.

Promoters commonly used in recombinant DNA construction include the β -lactamase (penicillinase) and lactose promoter systems and other microbial promoters, such as the T7 promoter system as described in U.S. Patent No. 4,952,496. Details concerning the nucleotide sequences of promoters are known, enabling a skilled worker to ligate them functionally with genes. The particular promoter used will generally be a matter of choice depending upon the desired results. Hosts that are appropriate for expression of the transferrin receptor genes, fragments, analogs or variants thereof, may include *E. coli*, *Bacillus* species, *Haemophilus*, fungi, yeast, *Moraxella*, *Bordetella*, or the baculovirus expression system may be used.

In accordance with this invention, it is preferred to make the transferrin receptor protein, fragment or analog thereof, by recombinant methods, particularly since the naturally occurring TfR protein as purified from a culture of a species of *Moraxella* may include trace amounts of toxic materials or other contaminants.

This problem can be avoided by using recombinantly produced TfR protein in heterologous systems which can be isolated from the host in a manner to minimize contaminants in the purified material. Particularly desirable hosts for expression in this regard include Gram positive bacteria which do not have LPS and are, therefore, endotoxin free. Such hosts include species of *Bacillus* and may be particularly useful for the

production of non-pyrogenic transferrin receptor, fragments or analogs thereof. Furthermore, recombinant methods of production permit the manufacture of Tbp1 or Tbp2 or respective analogs or fragments thereof, separate from one another which is distinct from the normal combined proteins present in *Moraxella*.

Biological Deposits

Certain vectors that contain at least a portion coding for a transferrin receptor protein from strains of *Moraxella catarrhalis* strain 4223 and Q8 and a strain of *M. catarrhalis* RH408 that are described and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 12301 Parklawn Drive, Rockville, Maryland, USA, pursuant to the Budapest Treaty and prior to the filing of this application. Samples of the deposited vectors and bacterial strain will become available to the public and the restrictions imposed on access to the deposits will be removed upon grant of a patent based upon this United States patent application. In addition, the deposit will be replaced if viable samples cannot be dispensed by the Depository. The invention described and claimed herein is not to be limited in scope by the biological materials deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or similar vectors or strains that encode similar or equivalent antigens as described in this application are within the scope of the invention.

Deposit Summary

DEPOSIT	ATCC DESIGNATION	DATE DEPOSITED
Phage LEM3-24	97,381	December 4, 1995
Phage SLRD-A	97,380	December 4, 1995
Plasmid pLEM29	97,461	March 8, 1996
Plasmid pSLRD35A	97,833	January 13, 1997
Plasmid pLEM37	97,834	January 13, 1997
Strain RH408	55,637	December 9, 1994

EXAMPLES

5 The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit
10 the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of
15 limitations.

 Methods of molecular genetics, protein biochemistry and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of
20 those skilled in the art.

Example 1

 This Example illustrates the preparation and immunization of guinea pigs with Tbp1 and Tbp2 proteins
25 from *M. catarrhalis*.

Tbp1 and Tbp2 proteins were obtained as follows:

Iron-starved crude total membrane preparations were diluted to 4 mg protein/ml in 50 mM Tris.HCl-1M NaCl, pH 8, in a total volume of 384 ml. Membranes were solubilized by the addition of 8 ml each of 0.5M EDTA and 30% sarkosyl and samples were incubated for 2 hours at room temperature, with gentle agitation. Solubilized membranes were centrifuged at 10K rpm for 20 min. 15 ml of apo-hTf-Sepharose 4B were added to the supernatant, and incubated for 2 hours at room temperature, with gentle shaking. The mixture was added into a column. The column was washed with 50 ml of 50mM Tris.HCl-1 M NaCl-250mM guanidine hydrochloride, to remove contaminating proteins. Tbp2 was eluted from the column by the addition of 100 ml of 1.5M guanidine hydrochloride. Tbp1 was eluted by the addition of 100 ml of 3M guanidine hydrochloride. The first 20 ml fractions were dialyzed against 3 changes of 50 mM Tris.HCl, pH 8.0. Samples were stored at -20°C, or dialyzed against ammonium bicarbonate and lyophilized.

Guinea pigs (Charles River) were immunized intramuscularly on day +1 with a 10 µg dose of Tbp1 or Tbp2 emulsified in complete Freund's adjuvant. Animals were boosted on days +14 and +29 with the same dose of protein emulsified in incomplete Freund's adjuvant. Blood samples were taken on day +42, and sera were used for analysis of bactericidal antibody activity. In addition, all antisera were assessed by immunoblot analysis for reactivity with *M. catarrhalis* 4223 proteins.

The bactericidal antibody activity of guinea pig anti-*M. catarrhalis* 4223 Tbp1 or Tbp2 antisera was determined as follows. A non-clumping *M. catarrhalis* strain RH408, derived from isolate 4223, was inoculated into 20 ml of BHI broth, and grown for 18 hr at 37°C, shaking at 170 rpm. One ml of this culture was used to

inoculate 20 ml of BHI supplemented with 25 mM ethylenediamine-di-hydroxyphenylacetic acid (EDDA; Sigma). The culture was grown to an OD₆₀₀ of 0.5. The cells were diluted 1:200,000 in 140 mM NaCl, 93mM NaHCO₃, 2mM Na barbiturate, 4mM barbituric acid, 0.5mM MgCl₂·6H₂O, 0.4mM CaCl₂·2H₂O, pH 7.6 (Veronal buffer), containing 0.1% bovine serum albumin (VBS) and placed on ice. Guinea pig anti-*M. catarrhalis* 4223 Tbp1 or Tbp2 antisera, along with prebleed control antisera, were heated to 56°C for 30 min. to inactivate endogenous complement. Serial twofold dilutions of each antisera in VBS were added to the wells of a 96-well Nunclon microtitre plate (Nunc, Roskilde, Denmark). Dilutions started at 1:8, and were prepared to a final volume of 25 µL in each well. 25 µL of diluted bacterial cells were added to each of the wells. A guinea pig complement (Biowhittaker, Walkersville, MD) was diluted 1:10 in VBS, and 25 µL portions were added to each well. The plates were incubated at 37°C for 60 min, gently shaking at 70 rpm on a rotary platform. 50 µL of each reaction mixture were plated onto Mueller Hinton (Becton-Dickinson, Cockeysville, MD) agar plates. The plates were incubated at 37°C for 72 hr and the number of colonies per plate were counted. Bactericidal titres were assessed as the reciprocal of the highest dilution of antiserum capable of killing greater than 50% of bacteria compared with controls containing pre-immune sera. Results shown in Table 1 below illustrate the ability of the anti-Tbp1 and anti-Tbp2 guinea pig antisera to lyse *M. catarrhalis*.

Example 2

This Example illustrates the preparation of chromosomal DNA from *M. catarrhalis* strains 4223 and Q8.

M. catarrhalis isolate 4223 was inoculated into 100 ml of BHI broth, and incubated for 18 hr at 37°C with

shaking. The cells were harvested by centrifugation at 10,000 x g for 20 min. The pellet was used for extraction of *M. catarrhalis* 4223 chromosomal DNA.

The cell pellet was resuspended in 20 ml of 10 mM Tris-HCl (pH 7.5)-1.0 mM EDTA (TE). Pronase and SDS were added to final concentrations of 500 µg/ml and 1.0%, respectively, and the suspension was incubated at 37°C for 2 hr. After several sequential extractions with phenol, phenol:chloroform (1:1), and chloroform:isoamyl alcohol (24:1), the aqueous extract was dialysed, at 4°C, against 1.0 M NaCl for 4 hr, and against TE (pH 7.5) for a further 48 hr with three buffer changes. Two volumes of ethanol were added to the dialysate, and the DNA was spooled onto a glass rod. The DNA was allowed to air-dry, and was dissolved in 3.0 ml of water. Concentration was estimated, by UV spectrophotometry, to be about 290 µg/ml.

M. catarrhalis strain Q8 was grown in BHI broth as described in Example 1. Cells were pelleted from 50 ml of culture by centrifugation at 5000 rpm for 20 minutes, at 4°C. The cell pellet was resuspended in 10 ml of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and proteinase K and SDS were added to final concentrations of 500 µg/ml and 1%, respectively. The sample was incubated at 37°C for 4 hours until a clear lysate was obtained. The lysate was extracted twice with Tris-saturated phenol/chloroform (1:1), and twice with chloroform. The final aqueous phase was dialysed for 24 hours against 2 X 1000 ml of 1 M NaCl at 4°C, changing the buffer once, and for 24 hours against 2 x 1000 ml of TE at 4°C, changing the buffer once. The final dialysate was precipitated with two volume of 100% ethanol. The DNA was spooled, dried and resuspended in 5 to 10 ml of TE buffer.

Example 3

This Example illustrates the construction of *M.*

catarrhalis chromosomal libraries in EMBL3.

A series of *Sau*3A restriction digests of chromosomal DNA, in final volumes of 10 μ L each, were carried out in order to optimize the conditions necessary to generate maximal amounts of restriction fragments within a 15 to 23 kb size range. Using the optimized digestion conditions, a large-scale digestion was set up in a 100 μ L volume, containing the following:

50 μ L of chromosomal DNA (290 μ g/ml), 33 μ L water, 10 μ L 10X *Sau*3A buffer (New England Biolabs), 1.0 μ L BSA (10 mg/ml, New England Biolabs), and 6.3 μ L *Sau*3A (0.04 U/ μ L). Following a 15 min. incubation at 37°C, the digestion was terminated by the addition of 10 μ L of 100 mM Tris-HCl (pH 8.0)-10 mM EDTA-0.1% bromophenol blue-50% glycerol (loading buffer). Digested DNA was electrophoresed through a 0.5% agarose gel in 40 mM Tris acetate-2 mM Na₂EDTA.2H₂O (pH 8.5) (TAE buffer) at 50 V for 6 hr. The region containing restriction fragments within a 15 to 23 kb molecular size range was excised from the gel, and placed into dialysis tubing containing 3.0 ml of TAE buffer. DNA was electroeluted from the gel fragment by applying a field strength of 1.0 V/cm for 18 hr. Electroeluted DNA was extracted once each with phenol and phenol:chloroform (1:1), and precipitated with ethanol. The dried DNA was dissolved in 5.0 μ L water.

Size-fractionated chromosomal DNA was ligated with *Bam*HI-digested EMBL3 arms (Promega), using T4 DNA ligase in a final volume of 9 μ L. The entire ligation mixture was packaged into lambda phage using a commercial packaging kit (Amersham), following manufacturer's instructions.

The packaged DNA library was amplified on solid media. 0.1 ml aliquots of *Escherichia coli* strain NM539 in 10 mM MgSO₄ (OD₆₀₀ = 0.5) were incubated at 37°C for 15

min. with 15 to 25 μ L of the packaged DNA library. Samples were mixed with 3 ml of 0.6% agarose containing 1.0% BBL trypticase peptone-0.5% NaCl (BBL top agarose), and mixtures were plated onto 1.5% agar plates containing 1.0% BBL trypticase peptone-0.5% NaCl, and incubated at 37°C for 18 hr. 3 ml quantities of 50 mM Tris-HCl (pH 7.5)-8 mM magnesium sulfate heptahydrate-100 mM NaCl-0.01% (w/v) gelatin (SM buffer) were added to each plate, and plates were left at 4°C for 7 hr. SM buffer containing phage was collected from the plates, pooled together, and stored in a screwcap tube at 4°C, with chloroform.

Chromosomal DNA from *M. catarrhalis* strain Q8 was digested with Sau3A I (0.1 unit/30 μ g DNA) at 37°C for 30 minutes and size-fractionated on a 0.6% low melting point agarose gel. DNA fragments of 15-23 kb were excised and the DNA was electroeluted for 25 minutes in dialysis tubing containing TAE (40 mM Tris acetate pH 8.5, 2 mM EDTA) at 150 V. The DNA was extracted once with phenol/chloroform (1:1), precipitated, and resuspended in water. The DNA was ligated overnight with EMBL3 BamH I arms (Promega) and the ligation mixture was packaged using the Lambda *in vitro* packaging kit (Stratagene) and plated onto *E. coli* LE392 cells. The library was titrated and stored at 4°C in the presence of 0.3% chloroform.

Example 4

This Example illustrates screening of the *M. catarrhalis* libraries.

Ten μ L aliquots of phage stock from the EMBL3/4223 sample prepared in Example 3 above were combined each with 100 μ L of *E. coli* strain LE392 in 10 mM MgSO₄ (OD₆₀₀ = 0.5) (plating cells), and incubated at 37°C for 15 min.

The samples were mixed with 3 ml each of BBL top agarose, and the mixtures were poured onto 1.5% agarose

plates containing 1% bacto tryptone-0.5% bacto yeast extract-0.05% NaCl (LB agarose; Difco) and supplemented with 200 μ M EDDA. The plates were incubated at 37°C for 18 hr. Plaques were lifted onto nitrocellulose filters (Amersham Hybond-C Extra) using a standard protocol, and the filters were immersed into 5% bovine serum albumin (BSA; Boehringer) in 20 mM Tris-HCl (pH 7.5)-150 mM NaCl (TBS) for 30 min at room temperature, or 4°C overnight. Filters were incubated for at least 1 hr at room temperature, or 18 hr at 4°C, in TBS containing a 1/1000 dilution of guinea pig anti-*M. catarrhalis* 4223 Tbp1 antiserum. Following four sequential 10 min. washes in TBS with 0.05% Tween 20 (TBS-Tween), filters were incubated for 30 min. at room temperature in TBS-Tween containing a 1/4000 dilution of recombinant Protein G labelled with horseradish peroxidase (rProtein G-HRP; Zymed). Filters were washed as above, and submerged into CN/DAB substrate solution (Pierce). Color development was arrested by immersing the filters into water. Positive plaques were cored from the plates, and each placed into 0.5 ml of SM buffer containing a few drops of chloroform. The screening procedure was repeated two more times, until 100% of the lifted plaques were positive using the guinea pig anti-*M. catarrhalis* 4223 Tbp1 antiserum.

The EMBL3/Q8 library was plated onto LE392 cells on YT plates using 0.7% top agar in YT as overlay. Plaques were lifted onto nitrocellulose filters and the filters were probed with oligonucleotide probes labelled with 32 P α -dCTP (Random Primed DNA labeling kit, Boehringer Mannheim). The pre-hybridization was performed in sodium chloride/sodium citrate (SSC) buffer (ref. 27) at 37°C for 1 hour and the hybridization was performed at 42°C overnight. The probes were based upon an internal sequence of 4223 *tbpA*:

I R D L T R Y D P G

(Seq ID No. 31)

4236-RD 5' ATTCGAGACTTAACACGCTATGACCCTGGC 3'

(Seq ID No 32)

5 4237-RD 5' ATTCGTGATTTAACCTCGCTATGACCCTGGT 3'

(Seq ID No 33).

Putative plaques were re-plated and submitted to second and third rounds of screening using the same procedures.

10 Phage clone SLRD-A was used to subclone the *tfr* genes for sequence analysis.

Example 5

15 This Example illustrates immunoblot analysis of the phage lysates using anti-*M. catarrhalis* 4223 Tbp1 and Tbp2 antisera.

Proteins expressed by the phage eluants selected in Example 4 above were precipitated as follows. 60 µL of each phage eluant were combined with 200 µL *E. coli* LE392 plating cells, and incubated at 37°C for 15 min. 20 The mixture was inoculated into 10 ml of 1.0% NZamine A-0.5% NaCl-0.1% casamino acids-0.5% yeast extract-0.2% magnesium sulfate heptahydrate (NZCYM broth), supplemented with 200 mM EDDA, and grown at 37°C for 18 hr, with shaking. DNase was added to 1.0 ml of the 25 culture, to a final concentration of 50 µg/ml, and the sample was incubated at 37°C for 30 min. Trichloroacetic acid was added to a final concentration of 12.5%, and the mixture was left on ice for 15 min. Proteins were pelleted by centrifugation at 13,000 x g 30 for 10 min, and the pellet was washed with 1.0 ml of acetone. The pellet was air-dried and resuspended in 50 µL 4% SDS-20 mM Tris- HCl (pH 8.0)-0.2 mM EDTA (lysis buffer).

35 Following SDS-PAGE electrophoresis through an 11.5% gel, the proteins were transferred to Immobilon-P

filters (Millipore) at a constant voltage of 20 V for 16 hr, in 25mM Tris-HCl, 220mM glycine-20% methanol (transfer buffer). Membranes were blocked in 5% BSA in TBS for 30 min. at room temperature. Blots were exposed either to guinea pig anti-*M. catarrhalis* 4223 Tbp1, or to guinea pig anti-*M. catarrhalis* 4223 Tbp2 antiserum, diluted 1/500 in TBS-Tween, for 2 hr at room temperature. Following three sequential 10 min. washes in TBS-Tween, membranes were incubated in TBS-Tween containing a 1/4000 dilution of rProtein G-HRP for 30 min. at room temperature. Membranes were washed as described above, and immersed into CN/DAB substrate solution. Color development was arrested by immersing blots into water.

Three EMBL3 phage clones expressed both a 115 kDa protein which reacted with anti-Tbp1 antiserum, and an 80 kDa protein, which reacted with anti-Tbp2 antiserum on Western blots and were thus concluded to contain genes encoding the transferrin receptor proteins of *Moraxella catarrhalis*.

Example 6

This Example illustrates the subcloning of the *M. catarrhalis* 4223 Tbp1 protein gene, *tbpA*.

Plate lysate cultures of the recombinant phage described in Example 5 were prepared by combining phage eluant and *E. coli* LE392 plating cells, to produce confluent lysis on LB agar plates. Phage DNA was extracted from the plate lysates using a Wizard Lambda Preps DNA Purification System (Promega), according to manufacturer's instructions.

The EMBL3 clone LM3-24 was found to contain a 13.2 kb insert, flanked by two *SalI* sites. A probe to a *tbpA* gene was prepared and consisted of a 300 base pair amplified product generated by PCR using two degenerate oligonucleotide primers corresponding to an amino acid sequence of part of the Tbp1 protein (Figure 1). The

primer sequences were based upon the amino acid sequences NEVTGLG (SEQ ID No: 17) and GAINEIE (SEQ ID No: 18), which had been found to be conserved among the deduced amino acid sequences from several different *N. meningitidis* and *Haemophilus influenzae* *tbpA* genes. The amplified product was cloned into pCRII (Invitrogen, San Diego, CA) and sequenced. The deduced amino acid sequence shared homology with other putative amino acid sequences derived from *N. meningitidis* and *H. influenzae* *tbpA* genes (Figure 12). The subclone was linearized with *NotI* (New England Biolabs), and labelled using a digoxigenin random-labelling kit (Boehringer Mannheim), according to manufacturer's instructions. The concentration of the probe was estimated to be 2 ng/ μ L.

DNA from the phage clone was digested with *HindIII*, *AvrII*, *SalI/SphI*, or *SalI/AvrII*, and electrophoresed through a 0.8% agarose gel. DNA was transferred to a nylon membrane (Genescreen Plus, Dupont) using an LKB VacuGene XL vacuum transfer apparatus (Pharmacia). Following transfer, the blot was air-dried, and pre-hybridized in 5X SSC-0.1% N-lauroylsarcosine-0.02% sodium dodecyl sulfate-1.0% blocking reagent (Boehringer Mannheim) in 10 mM maleic acid-15 mM NaCl (pH 7.5) (pre-hybridization solution). Labelled probe was added to the pre-hybridization solution to a final concentration of 6 ng/ml, and the blot was incubated in the probe solution at 42°C for 18 hr. The blot was washed twice in 2X SSC-0.1% SDS, for 5 min. each at room temperature, then twice in 0.1X SSC-0.1% SDS for 15 min. each at 60°C. Following the washes, the membrane was equilibrated in 100mM maleic acid-150 mM NaCl (pH 7.5) (buffer 1) for 1 min, then left in 1.0% blocking reagent (Boehringer Mannheim) in buffer 1 (buffer 2) for 60 min, at room temperature. The blot was exposed to anti-DIG-alkaline phosphatase (Boehringer Mannheim) diluted 1/5000 in buffer 2, for 30 min. at room temperature.

Following two 15 min. washes in buffer 1, the blot was equilibrated in 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 50 mM MgCl₂ (buffer 3) for 2 min. The blot was wetted with Lumigen PPD substrate (Boehringer-Mannheim),
5 diluted 1/100 in buffer 3, then wrapped in Saran wrap, and exposed to X-ray film for 30 min. The probe hybridized to a 3.8 kb *Hind*III-*Hind*III, a 2.0 kb *Avr*II-*Avr*II, and a 4.2 kb *Sal*I-*Sph*I fragment.

In order to subclone the 3.8 kb *Hind*III-*Hind*III
10 fragment into pACYC177, phage DNA from the EMBL3 clone, and plasmid DNA from the vector pACYC177 (New England Biolabs), were digested with *Hind*III, and fractionated by electrophoresis on a 0.8% agarose gel. The 3.8 kb
15 *Hind*III-*Hind*III phage DNA fragment, and the 3.9 kb *Hind*III-*Hind*III pACYC177 fragment, were excised from the gel and purified using a Geneclean kit (Bio 101 Inc., LaJolla, CA), according to manufacturer's directions. Purified insert and vector were ligated together using
20 T4 DNA ligase (New England Biolabs), and transformed into *E. coli* HB101 (Gibco BRL). A Qiagen Plasmid Midi-Kit (Qiagen) was used to extract and purify sequencing-quality DNA from one of the ampicillin-resistant/kanamycin-sensitive transformants, which was
25 found to carry a 3.8 kb *Hind*III-*Hind*III insert. The subclone was named pLEM3. As described in Example 7, below, subsequent sequencing revealed that pLEM3 contained the first about 2.0 kb of *tbpA* sequence (Figures 2 and 5).

In order to subclone the remaining 1 kb of the *tbpA*
30 gene, a 1.6 kb *Hind*III-*Hind*III fragment was subcloned into pACYC177 as described above, and transformed by electroporation into *E. coli* HB101 (Gibco BRL). A Midi-Plasmid DNA kit (Qiagen) was used to extract plasmid DNA from a putative kanamycin-sensitive transformant
35 carrying a plasmid with a 1.6 kb *Hind*III-*Hind*III insert. The subclone was termed pLEM25. As described in

Example 7 below, sequencing revealed that pLEM25 contained the remaining 1 kb of the *tbpA* gene (Figure 2 and 5).

Example 7

5 This Example illustrates the subcloning of the *M. catarrhalis* 4223 *tbpB* gene.

As described above, in all *Neisseriae* and *Haemophilus* species examined prior to the present invention, *tbpB* genes have been found immediately
10 upstream of the *tbpA* genes which share homology with the *tbpA* gene of *M. catarrhalis* 4223. However, the sequence upstream of *M. catarrhalis* 4223 did not correspond with other sequences encoding *tbpB*.

In order to localize the *tbpB* gene within the EMBL3
15 phage clone, a Southern blot was carried out using a degenerate probe from a highly conserved amino acid region within the Tbp2 protein. A degenerate oligonucleotide probe, was designed corresponding to the sequence encoding EGGFYGP (SEQ ID No: 30), which is
20 conserved within the Tbp2 protein in a variety of *Neisseriae* and *Haemophilus* species. The probe was labelled with digoxigenin using an oligonucleotide tailing kit (Boehringer Mannheim), following the manufacturer's instructions. *HindIII* - digested EMBL3
25 clone DNA was fractionated through a 0.8% agarose gel, and transferred to a GeneClean Plus nylon membrane as described in Example 6. Following hybridization as described above, the membrane was washed twice in 2X SSC-0.1% SDS, for 5 min. each at room temperature, then
30 twice in 0.1X SSC-0.1% SDS for 15 min. each, at 50°C. Detection of the labelled probe was carried out as described above. The probe hybridized to a 5.5 kb *NheI*-*SalI* fragment.

The 5.5 kb *NheI*-*SalI* fragment was subcloned into
35 pBR328 as follows. LEM3-24 DNA, and pBR328 DNA, were digested with *NheI*-*SalI*, and electrophoresed through

0.8% agarose. The 5.5 kb *NheI-SalI* fragment, and the 4.9 kb pBR328 *NheI-SalI* fragments were excised from the gel, and purified using a Geneclean kit as described in Example 6. The fragments were ligated together using T4 DNA ligase, and transformed into *E. coli* DH5. A Midi-Plasmid DNA kit (Qiagen) was used to extract DNA from an ampicillin resistant / tetracycline sensitive clone containing a 5.5 kb *NheI-SalI* insert. This subclone was termed pLEM23. Sequencing revealed that pLEM23 contained 2 kb of the *tbpB* gene from *M. catarrhalis* 4223 (Figure 2).

Example 8:

This Example illustrates the subcloning of *M. catarrhalis* Q8 *tfr* genes.

The *M. catarrhalis* Q8 *tfr* genes were subcloned as follows. Phage DNA was prepared from plates. Briefly, the top agarose layer from three confluent plates was scraped into 9 ml of SM buffer (0.1 M NaCl, 0.2% MgSO₄, 50 mM Tris-HCl, pH 7.6, 0.01% gelatin) and 100 µl of chloroform was added. The mixture was vortexed for 10 sec, then incubated at room temperature for 2h. The cell debris was removed by centrifugation at 8000 rpm for 15 min at 4°C in an SS34 rotor (Sorvall model RC5C). The phage was pelleted by centrifugation at 35,000 rpm in a 70.1 Ti rotor at 10°C for 2h (Beckman model L8-80) and was resuspended in 500 µl of SM buffer. The sample was incubated at 4°C overnight, then RNase and DNase were added to final concentrations of 40 µg/ml and 10 µg/ml, respectively and the mixture incubated at 37°C for 1h. To the mixture were added 10 µl of 0.5 M EDTA and 5 µl of 10% SDS and the sample was incubated at 6°C for 15 min. The mixture was extracted twice with phenol/chloroform (1:1) and twice with chloroform and the DNA was precipitated by the addition of 2.5 volumes of absolute ethanol.

A partial restriction map was generated and fragments were subcloned using the external Sal I sites from EMBL3 and internal AvrII or EcoR I sites as indicated in Figure 4. In order to facilitate the subcloning, plasmid pSKMA was constructed which introduces a novel multiple cloning site into pBluescript.SK (Stratagene). Oligonucleotides were used to introduce restriction sites for Mst II, Sfi I, and Avr II between the Sal I and Hind III sites of pBluescript.SK:

	Sal I	Cla I	Mst II	Sfi I	Avr II	Hind III
	↓	↓	↓	↓	↓	↓
15	4639-RD	5'	TCGACGGTAT	CGATGGCC	TTAG	GGGC CTAGGA 3'
	(SEQ ID No: 34)					
	4640-RD	3'	GCCATA	GCTACCGG	AATC	CCCG GATCCTTCGA
	(SEQ ID No: 35)					

Plasmid pSLRD1 contains a ~1.5 kb Sal I-Avr II fragment cloned into pSKMA; plasmids pSLRD2 and pSLRD4 contain ~2 kb and 4 kb AvrII-AvrII fragments cloned into pSKMA, respectively and contain the complete *tbpA* gene. Plasmid pSLRD3 contains a ~2.3 kb AvrII-EcoR I fragment cloned into pSKMA and plasmid SLRD5 is a 22.7 kb EcoRI - EcoRI fragment cloned into pSKMA. These two clones contain the complete *tbpB* gene (Figure 7).

Example 9

This Example illustrates sequencing of the *M. catarrhalis* *tbp* genes.

Both strands of the *tbp* genes subcloned according to Examples 6 to 8 were sequenced using an Applied Biosystems DNA sequencer. The sequences of the *M. catarrhalis* 4223 and Q8 *tbpA* genes are shown in Figures 5 and 10 respectively. A derived amino acid sequence was compared with other Tbp1 amino acid sequences, including

those of *Neisseriae meningitidis*, *Neisseriae gonorrhoeae*, and *Haemophilus influenzae* (Figure 12). The sequence of the *M. catarrhalis* 4223 and Q8 *tbpB* genes are shown in Figures 6 and 11 respectively. In order to obtain sequence from the putative beginning of the *tbpB* gene of *M. catarrhalis* 4223, sequence data were obtained directly from the clone LEM3-24 DNA. This sequence was verified by screening clone DS-1754-1. The sequence of the translated *tbpB* genes from *M. catarrhalis* 4223 and Q8 shared homology with deduced Tbp2 amino acid sequences of *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae* (Figure 13).

Example 10

This Example illustrates the generation of an expression vector to produce recombinant Tbp1 protein. The construction scheme is shown in Figure 14.

Plasmid DNA from subclone pLEM3, prepared as described in Example 6, was digested with *Hind*III and *Bgl*II to generate a 1.84 kb *Bgl*II-*Hind*III fragment, containing approximately two-thirds of the *tbpA* gene. *Bam*HI was added to the digest to eliminate a comigrating 1.89kb *Bgl*II-*Hind*III vector fragment. In addition, plasmid DNA from the vector pT7-7 was digested with *Nde*I and *Hind*III. To create the beginning of the *tbpA* gene, an oligonucleotide was synthesized based upon the first 61 bases of the *tbpA* gene to the *Bgl*II site; an *Nde*I site was incorporated into the 5' end. Purified insert, vector and oligonucleotide were ligated together using T4 ligase (New England Biolabs), and transformed into *E. coli* DH5 α . DNA was purified from one of the 4.4 kb ampicillin-resistant transformants containing correct restriction sites (pLEM27).

Purified pLEM27 DNA was digested with *Hind*III, ligated to the 1.6 kb *Hind*III-*Hind*III insert fragment

of pLEM25 prepared as described in Example 6, and transformed into *E. coli* DH5 α . DNA was purified from an ampicillin-resistant transformant containing the correct restriction sites (pLEM29), and was transformed by electroporation into BL21 (DE3) (Novagen; Madison, WI) to produce *E. coli* pLEM29B-1.

A single isolated transformed colony was used to inoculate 100 ml of YT broth containing 100 μ g/ml ampicillin, and the culture was grown at 37°C overnight, shaking at 200 rpm. 200 μ l of the overnight culture were inoculated into 10 ml of YT broth containing 100 μ g/ml ampicillin, and the culture was grown at 37°C to an OD₅₇₈ of 0.35. The culture was induced by the addition of 30 μ l of 100 mM IPTG, and the culture was grown at 37°C for an additional 3 hours. One ml of culture was removed at the time of induction (t=0), and at t=1 hr and t=3 hrs. One ml samples were pelleted by centrifugation, and resuspended in 4%SDS-20 mM Tris.Cl, pH 8-200 μ M EDTA (lysis buffer). Samples were fractionated on an 11.5% SDS-PAGE gel, and transferred onto Immobilon filters (Amersham). Blots were developed using anti-Tbpl (*M. catarrhalis* 4223) antiserum, diluted 1:1000, as the primary antibody, and rproteinG conjugated with horseradish peroxidase (Zymed) as the secondary antibody. A chemiluminescent substrate (Lumiglo; Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used for detection. Induced recombinant proteins were visible on the Coomassie-stained gels (Fig 15). The anti-Tbpl (4223) antiserum recognized the recombinant proteins on Western blots.

Example 11

This Example illustrates the extraction and purification of recombinant Tbpl of *M. catarrhalis* 4223. Recombinant Tbpl protein, which is contained in inclusion bodies, was purified from *E. coli* cells

expressing the *tbpA* gene (Example 10), by a procedure as shown in Figure 16. *E. coli* cells from a 500 ml culture, prepared as described in Example 10, were resuspended in 50 ml of 50 mM Tris-HCl, pH 8.0 containing 0.1 M NaCl and 5 mM AEBSF (protease inhibitor), and disrupted by sonication (3 x 10 min. 70% duty circle). The extract was centrifuged at 20,000 x g for 30 min. and the resultant supernatant which contained > 85% of the soluble proteins from *E. coli* was discarded.

The remaining pellet (Figure 16, PPT₁) was further extracted in 50 ml of 50 mM Tris, pH 8.0 containing 0.5% Triton X-100 and 10 mM EDTA. After centrifugation at 20,000 x g for 30 min., the supernatant containing residual soluble proteins and the majority of the membrane proteins was discarded.

The remaining pellet (Figure 16, PPT₂) was further extracted in 50 ml of 50 mM Tris, pH 8.0 containing 2M urea and 5 mM dithiothreitol (DTT). After centrifugation at 20,000 x g for 30 min., the resultant pellet (Figure 16, PPT₃) obtained after the above extraction contained the purified inclusion bodies.

The Tbp1 protein was solubilized from PPT₃ in 50 mM Tris, pH 8.0, containing 6 M guanidine hydrochloride and 5 mM DTT. After centrifugation, the resultant supernatant was further purified on a Superdex 200 gel filtration column equilibrated in 50 mM Tris, pH 8.0, containing 2M guanidine hydrochloride and 5 mM DTT. The fractions were analyzed by SDS-PAGE and those containing purified Tbp1 were pooled. Triton X-100 was added to the pooled Tbp1 fraction to a final concentration of 0.1%. The fraction was then dialyzed overnight at 4°C against 50 mM Tris, pH 8.0 and then centrifuged at 20,000 x g for 30 min. The protein remained soluble under these conditions and the purified Tbp1 was stored at -20° C. The purification procedure shown in Figure

16 produced Tbp1 protein that was at least 70% pure as determined by SDS-PAGE analysis (Figure 17).

Example 12

5 This Example illustrates the construction of an expression plasmid for rTbp2 of *M. catarrhalis* 4223 without a leader sequence.

The construction scheme for the plasmid expressing rTbp2 is shown in Figure 18. Oligonucleotides were used to construct the first approximately 58 bp of the
10 *M. catarrhalis* 4223 *tbpB* gene encoding the mature protein. An *NdeI* site was incorporated into the 5' end of the oligonucleotides:

5'TATGTGTGGTGGCAGTGGTGGTTCAAATCCACCTGCTCCTACGCCCATT
15 CCAAATG (SEQ ID NO: 36) 3'
3'ACACACCACCGTCACCACCAAGTTTAGGTGGACGAGGATGCGGGTAAGG
TTTACGATC (SEQ ID NO: 37) 5'

An *NheI*-*ClaI* fragment, containing approximately 1kb of
20 the *tbpB* gene from pLEM23, prepared as described in Example 7, was ligated to the above oligonucleotides and inserted into pT7-7 cut with *NdeI*-*ClaI*, generating pLEM31, which thus contains the 5'-half of *tbpB*. Oligonucleotides also were used to construct the last
25 approximately 104 bp of the *tbpB* gene, from the *AvaII* site to the end of the gene. A *BamHI* site was incorporated into the 3' end of the oligonucleotides:

5'GTCCAAATGCAAACGAGATGGGCGGGTCATTTACACACAACGCCGATG
30 ACAGCAAAGCCTCTGTGGTCTTTGGCACAAAAGACAACAAGAAGTTAAGTAGTA
G (SEQ ID NO: 38) 3'

3'GTTTACGTTTGCTCTACCCGCCCCAGTAAATGTGTGTTGCGGCTACTGTC
GTTTCGGAGACACCAGAAACCGTGTTTTTCTGTTGTTCTTCAATTCATCATCCTAG
35 (SEQ ID NO: 39) 5'

A *Cla*I-*Ava*II fragment from pLEM23, containing approximately 0.9 kb of the 3'-end of the *tbpB* gene, was ligated to the *Ava*II-*Bam*HI oligonucleotides, and inserted into pT7-7 cut with *Cla*I-*Bam*HI, generating
5 pLEM32. The 1.0 kb *Nde*I-*Cla*I insert from pLEM31 and the 1.0 kb *Cla*I-*Bam*HI insert from pLEM32 were then inserted into pT7-7 cut with *Nde*I-*Bam*HI, generating pLEM33 which has a full-length *tbpB* gene under the direction of the T7 promoter.

10 DNA was purified from pLEM33 and transformed by electroporation into electrocompetent BL21(DE3) cells (Novagen; Madison, WI), to generate strain pLEM33B-1. Strain pLEM33B-1 was grown, and induced using IPTG, as described above in Example 10. Expressed proteins were
15 resolved by SDS-PAGE and transferred to membranes suitable for immunoblotting. Blots were developed using anti-4223 Tbp2 antiserum, diluted 1:4000, as the primary antibody, and rprotein G conjugated with horseradish peroxidase (Zymed) as the secondary antibody. A
20 chemiluminescent substrate (Lumiglo; Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used for detection. Induced recombinant proteins were visible on the Coomassie blue-stained gels (Fig. 19). The anti-4223 Tbp2 antiserum recognized the recombinant proteins
25 on Western blots.

Example 13

This Example illustrates the generation of an expression plasmid for rTbp2 of *M. catarrhalis* 4223 with a leader sequence.

30 The construction scheme is shown in Figure 18. Oligonucleotides containing the natural leader sequence of the *M. catarrhalis* 4223 *tbpB* gene were used to construct the first approximately 115 bp of the *tbpB* gene to the *Nhe*I site. An *Nde*I site was incorporated
35 into the 5' end of the oligonucleotides:

5' TATGAAACACATTCCTTTAACCACACTGTGTGTGGCAATCTCTGCCGTC
TTATTAACCGCTTGTGGTGGCAGTGGTGGTTCAAATCCACCTGCTCCTACGCCCAT
TCCAAATG (SEQ ID NO: 40) 3'

5 3' ACTTTGTGTAAGGAAATTGGTGTGACACACACCGTTAGAGACGGCAGAA
TAATTGGCGAACACCACCGTCACCACCAAGTTTAGGTGGACGAGGATGCGGGTAAG
GTTTACGATC (SEQ ID NO: 41) 5'

10 The *NdeI*-*NheI* oligonucleotides were ligated to pLEM33
cut with *NdeI*-*NheI*, generating pLEM37, which thus
contains a full-length 4223 *tbpB* gene encoding the Tbp2
protein with its leader sequence, driven by the T7
promoter.

15 DNA from pLEM37 was purified and transformed by
electroporation into electrocompetent BL21(DE3) cells
(Novagen; Madison, WI), to generate strain pLEM37B-2.
pLEM37B-2 was grown, and induced using IPTG, as
described above in Example 10. Expressed proteins were
resolved by SDS-PAGE and transferred to membranes
20 suitable for immunoblotting. Blots were developed
using anti-4223 Tbp2 antiserum, diluted 1:4000, as the
primary antibody, and rprotein G conjugated with
horseradish peroxidase (Zymed) as the secondary
antibody. A chemiluminescent substrate (Lumiglo;
25 Kirkegaard and Perry Laboratories, Gaithersburg, MD)
was used for detection. Induced recombinant proteins
were visible on Coomassie-blue stained gels (Fig. 21).
The anti-4223 Tbp2 antiserum recognized the
recombinant proteins on Western blots.

30 **Example 14**

This Example illustrates the construction of an
expression plasmid for rTbp2 of *M. catarrhalis* Q8
without a leader sequence.

35 The construction scheme for rTbp2 is shown in
Figure 20. The 5'-end of the *tbpB* gene of *M.*
catarrhalis Q8 was PCR amplified from the Cys¹ codon of

the mature protein through the Bsm I restriction site.

An Nde I restriction site was introduced at the 5' end, for later cloning into pT7-7, and the final PCR fragment was 238 bp in length. The PCR primers are indicated below:

NdeI C G G S S G G F N

5' GAATTCCATATG TGT GGT GGG AGC TCT GGT GGT TTC AAT C

3' 5247.RD (SEQ ID No: 42)

5' CCCATGGCAGGTTCTTGAATGCCTGAAACT 3' 5236.RD
(SEQ ID No: 43).

The Q8 *tbpB* gene was subcloned in two fragments contained on plasmids SLRD3 and SLRD5, prepared as described in Example 8. Plasmid SLRD3-5 was constructed to contain the full-length *tbpB* gene by digesting SLRD5 with EcoR I and Dra I, which releases the 3'-end of *tbpB*, and inserting this ~ 619 bp fragment into SLRD3 which had been digested with EcoR I and Sma I. The 1.85 kb Bsm I-BamH I fragment from SLRD 3-5 was ligated with the 238 bp PCR fragment and inserted into pT7-7 that had been digested with Nde I and BamH I, generating plasmid SLRD35B. This plasmid thus contains the full-length *tbpB* gene without its leader sequence, under the direction of the T7 promoter. DNA from SLRD35B was purified and transformed by electroporation into electrocompetent BL21(DE3) cells to generate strain SLRD35BD which was grown and induced using IPTG, as described above in Example 10. Expressed proteins were resolved by SDS-PAGE and the induced Tbp2 protein was clearly visible by Coomassie blue staining (Fig. 19).

Example 15

This Example illustrates the generation of an expression plasmid for rTbp2 of *M. catarrhalis* Q8 with

a leader sequence.

The construction scheme for the rTbp2 is shown in Figure 20. The 5'-end of the Q8 *tbpB* gene was PCR amplified from the ATG start codon to the Bsm I restriction site. An Nde I site was engineered at the 5'-end, to facilitate cloning into the pT7-7 expression vector, and the final PCR fragment was 295 bp. The PCR primers are indicated below:

10 Nde I K H I P L T
5' GAATTCATATG AAA CAC ATT CCT TTA ACC 3' 5235.RD
(SEQ ID No: 44)

15 5' CCCATGGCAGGTTCTTGAATGCCTGAAACT 3' 5236.RD
(SEQ ID No: 43).

SLRD3-5 (Example 14) was digested with Bsm I and BamH I, generating a 1.85 kb fragment, which was ligated with the 295bp PCR fragment and ligated into pT7-7 that had been digested with Nde I and BamH I. The resulting plasmid SLRD35A thus contains the full-length Q8 *tbpB* gene with its endogenous leader sequence under the control of the T7 promoter. DNA from SLRD35A was purified and transformed by electroporation into electrocompetent BL21(DE3) cells to generate strain SLRD35AD which was grown and induced using IPTG, as described above in Example 10. Expressed proteins were resolved by SDS-PAGE and the induced Tbp2 protein was clearly visible by Coomassie blue staining (Fig. 19).

30 **Example 16**

This Example illustrates the extraction and purification of rTbp2 of *M. catarrhalis* 4223 and Q8 from *E. coli*.

35 pLEM37B (4223) and SLRD35AD (Q8) transformants were grown to produce Tbp2 in inclusion bodies and then the Tbp2 was purified according to the scheme in Figure

22. *E. coli* cells from a 500 mL culture, were resuspended in 50 mL of 50 mM Tris-HCl, pH 8.0 containing 5 mM AEBSF (protease inhibitor), and disrupted by sonication (3 x 10 min, 70% duty cycle).
5 The extract was centrifuged at 20,000 x *g* for 30 min and the resultant supernatant which contained > 95% of the soluble proteins from *E. coli* was discarded.

The remaining pellet (PPT₁) was further extracted in 50 mL of 50 mM Tris, pH 8.0 containing 0.5% Triton X-100 and 10 mM EDTA. The mixture was stirred at 4°C
10 for at least 2 hours and then centrifuged at 20,000 x *g* for 30 min and the supernatant containing residual soluble proteins and the majority of the membrane proteins was discarded.

15 The resultant pellet (PPT₂) obtained after the above extraction contained the inclusion bodies. The Tbp2 protein was solubilized in 50 mM Tris, pH 8.0, containing 6 M guanidine and 5 mM DTT. After centrifugation, the resultant supernatant was further
20 purified on a Superdex 200 gel filtration column equilibrated in 50 mM Tris, pH 8.0, containing 2 M guanidine and 5 mM DTT. The fractions were analyzed by SDS-PAGE and those containing purified Tbp2 were
25 pooled. Triton X-100 was added to the pooled Tbp2 fraction to a final concentration of 0.1%. The fraction was then dialyzed overnight at 4°C against PBS, and then centrifuged at 20,000 x *g* for 30 min. The protein remained soluble under these conditions and the
30 purified Tbp2 was stored at -20°C. Figure 22 shows the SDS PAGE analysis of fractions of the purification process for rTbp2 from strain 4223 (Panel A) and strain Q8 (Panel B). The rTbp2 was at least 70% pure.

Groups of five BALB/c mice were injected three times subcutaneously (s.c.) on days 1, 29 and 43 with
35 purified rTbp2 (0.3 mg to 10 mg) from *M. catarrhalis* strains 4223 and Q8 in the presence or absence of AlPO₄.

(1.5 mg per dose). Blood samples were taken on days 14, 28, 42 and 56 for analysing the anti-rTbp2 antibody titers by EIAs.

Groups of two rabbits and two guinea pigs (Charles River, Quebec) were immunized intramuscularly (i.m.) on day 1 with a 5 mg dose of purified rTbp2 protein emulsified in complete Freund's adjuvant (CFA). Animals were boosted on days 14 and 29 with the same dose of protein emulsified in incomplete Freund's adjuvant (IFA). Blood samples were taken on day 42 for analysing anti-rTbp2 antibody titers and bactericidal activity. Table 2 below shows the bactericidal activity of antibodies raised to the recombinant transferrin binding proteins rTbp1 (4223), rTbp2 (4223) and rTbp2 (Q8), prepared as described in these Examples, against *M. catarrhalis* strains 4223 and Q8.

Example 17

This Example illustrates the binding of Tbp2 to human transferrin *in vitro*.

Transferrin-binding activity of Tbp2 was assessed according to the procedures of Schryvers and Lee (ref. 28) with modifications. Briefly, purified rTbp2 was subjected to discontinuous electrophoresis through 12.5% SDS-PAGE gels. The proteins were electrophoretically transferred to PVDF membrane and incubated with horseradish peroxidase-conjugated human transferrin (HRP-human transferrin, 1:50 dilution) (Jackson ImmunoResearch Labs Inc., Mississauga, Ontario) at 4°C for overnight. LumiGLO substrate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was used for chemiluminescent detection of HRP activity according to the manufacturer's instructions. Both 4223 rTbp2 and Q8 rTbp2 bind to human transferrin under these conditions, as shown in Figure 24.

Example 18

This Example illustrates antigenic conservation of

Tbp2 amongst *M. catarrhalis* strains.

Whole cell lysates of *M. catarrhalis* strains and *E. coli* strains expressing recombinant Tbp2 proteins were separated by SDS-PAGE and electrophoretically transferred to PVDF membrane. Guinea pig anti-4223 rTbp2 or anti-Q8 rTbp2 antisera were used as first antibody and alkaline phosphatase conjugated goat anti-guinea pig antibody was used as second antibody to detect Tbp2. *M. catarrhalis* strains 3, 56, 135, 585, 4223, 5191, 8185 and ATCC 25240 were tested and all showed specific reactivity with anti-4223 rTbp2 or anti-Q8 rTbp2 antibody (Figure 25).

Table 3 illustrates the ability of anti-rTbp2 antibodies from one *M. catarrhalis* strain to recognize native or recombinant protein from a homologous or heterologous *M. catarrhalis* strain.

Example 19

This Example illustrates PCR amplification of the *tbpB* gene from *M. catarrhalis* strain R1 and characterization of the amplified R1 *tbpB* gene.

Chromosomal DNA from *M. catarrhalis* strain R1 was prepared using standard techniques. The design of the oligonucleotide sense primer was based on a region approximately 274 bases upstream of the *M. catarrhalis* 4223 *tbpB* gene, and the antisense primer was based upon a region approximately 11 bases downstream of the end of 4223 *tbpB*. The following primers were used:

sense primer (4940): 5' GATATAAGCACGCCCTACTT 3'
(SEQ ID No: 48)
antisense primer (4967): 5' CCCATCAGCCAAACAAACATTGTGT 3'
(SEQ ID No: 49).

Each reaction tube contained 10 mM Tris-HCl (pH 8.85), 25 mM KCl, 5 mM $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgSO_4 , 800 mM dNTPs, 1.0 mg each of primers 4940 and 4967, 10 ng of R1 DNA, and 2.5 U Pwo DNA polymerase (Boehringer

Mannheim) in a total volume of 100 μ l. The thermocycler was programmed for 5 min at 95°C, followed by 25 cycles of 95°C for 30 sec, 50°C for 45 sec, and 72°C for 2 min, and a 10 min final elongation
5 elongation at 72°C. The amplified product was purified using a Geneclean (BIO 101) according to the manufacturer's instructions, and sequenced.

A partial restriction map of *M. catarrhalis* strain R1 *tbpB* prepared as just described is shown in Figure
10 26. The nucleotide and deduced amino acid sequences of the PCR amplified R1 *tbpB* gene are shown in Figure 27. The R1 *tbpB* gene encodes a 714 amino acid protein of molecular weight 76.8 kDa. The leader sequence of the R1 Tbp2 protein is identical to that of the 4223 and Q8
15 Tbp2 proteins. When the deduced R1 Tbp2 sequence was aligned with the 4223 Tbp2 sequence, it was found to be 83% identical and 88% homologous (Fig. 28). The conserved LEGGFYG (SEQ ID No: 50) epitope was present, as found in Tbp2 from other *M. catarrhalis* strains as
20 well as the *H. influenzae* and *N. meningitidis* Tbp2 proteins.

SUMMARY OF THE DISCLOSURE

In summary of this disclosure, the present
25 invention provides purified and isolated DNA molecules containing transferrin receptor genes of *Moraxella catarrhalis*, the sequences of these transferrin receptor genes, and the derived amino acid sequences thereof. The genes and DNA sequences are useful for diagnosis,
30 immunization, and the generation of diagnostic and immunological reagents. Immunogenic compositions, including vaccines, based upon expressed recombinant Tbp1 and/or Tbp2, portions thereof, or analogs thereof, can be prepared for prevention of diseases caused by
35 *Moraxella*. Modifications are possible within the scope of this invention.

TABLE I

**BACTERICIDAL ANTIBODY TITRES FOR
M. CATARRHALIS ANTIGENS**

ANTIGEN ¹	SOURCE OF ANTISERA ²	BACTERICIDAL TITRE ³ RH408 ⁴		BACTERICIDAL TITRE Q8 ⁵	
		Pre-Immune	Post-Immune	Pre-Immune	Post-Immune
TBP1	GP	< 3.0	4.2-6.9	< 3.0	4.4.-6.2
TBP2	GP	< 3.0	12.0-13.6	< 3.0	< 3.0-4.0

- 1 antigens isolated from *M. catarrhalis* 4223
- 2 GP = guinea pig
- 3 bactericidal titres: expressed in log₂ as the dilution of antiserum capable of killing 50% of cells
- 4 *M. catarrhalis* RH408 is a non-clumping derivative of *M. catarrhalis* 4223
- 5 *M. catarrhalis* Q8 is a clinical isolate which displays a non-clumping phenotype

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TABLE 2

Antigen	Bactericidal titre - RH408		Bactericidal titre - Q8	
	pre-immune	post-immune	pre-immune	post-immune
rTbp1 (4223)	< 3.0	< 3.0	< 3.0	< 3.0
rTbp2 (4223)	< 3.0	10-15	< 3.0	< 3.0
rTbp2 (Q8)	NT	NT	< 3.0	5.5-7.5

Antibody titres are expressed in \log_2 as the dilution of antiserum capable of killing 50% of cells

NT = not tested

TABLE 3

ELISA titres for anti-rTbp2 antibodies recognizing native or rTbp2 from strain 4223 or rTbp2 from strain Q8

Coated antigen	Anti-rTbp2 (4223) Antibody Titres		Anti-rTbp2 (Q8) Antibody Titres	
	Rabbit antisera	Guinea pig antisera	Rabbit antisera	Guinea pig antisera
Native Tbp2 (4223)	409,600	1,638,400	25,600	51,200
	204,800	1,638,400	25,600	102,400
rTbp2 (4223)	409,600	1,638,400	102,400	204,800
	409,600	1,638,400	102,400	204,800
rTbp2 (Q8)	409,600	1,638,400	1,638,400	1,638,400
	102,400	1,638,400	409,600	1,638,400

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CLAIMS

What we claim is:

1. A purified and isolated nucleic acid molecule encoding a transferrin receptor protein of a strain of *Moraxella* or a fragment or an analog of the transferrin receptor protein.
2. The nucleic acid molecule of claim 1 wherein the transferrin receptor protein is the transferrin receptor binding protein 1 (Tbp1) of the *Moraxella* strain.
3. The nucleic acid molecule of claim 2 wherein the transferrin receptor protein is the transferrin receptor binding protein 2 (Tbp2) of the *Moraxella* strain.
4. The nucleic acid molecule of claim 1 wherein the strain of *Moraxella* is a strain of *Moraxella catarrhalis*.
5. The nucleic acid molecule of claim 4 wherein the strain of *Moraxella catarrhalis* is *Moraxella catarrhalis* 4223, Q8 or R1.
6. A purified and isolated nucleic acid molecule having a DNA sequence selected from the group consisting of:
 - (a) a DNA sequence as set out in Figure 5, 6, 10, 11 or 27 (SEQ ID Nos: 1, 2, 3, 4, 5, 6, 7, 8, 45 or 46) or the complementary DNA sequence thereto;
 - (b) a DNA sequence encoding an amino acid sequence as set out in Figure 5, 6, 10, 11 or 27 (SEQ ID Nos: 9, 10, 11, 12, 13, 14, 15, 16 or 47) or the complementary DNA sequence thereto; and
 - (c) a DNA sequence which hybridizes under stringent conditions to any one of the DNA sequences defined in (a) or (b).
7. The nucleic acid molecule of claim 6, wherein the DNA sequence defined in (c) has at least about 90% sequence identity with any one of the DNA sequences

defined in (a) or (b).

8. The nucleic acid molecule of claim 6 wherein the DNA sequence defined in (c) is that encoding the equivalent transferrin receptor protein from another strain of *Moraxella*.

9. A vector adapted for transformation of a host comprising the nucleic acid molecule of claim 1 or 6.

10. The vector of claim 9 encoding a fragment of a transferrin receptor protein and having the characteristics of a plasmid selected from the group consisting of pLEM3, pLEM25, pLEM23, DS-1698-1-1, DS-1754-1, pSLRD2, pSLRD3, pSLRD4 and pSLRD5.

11. The vector of claim 9 further comprising expression means operatively coupled to the nucleic acid molecule for expression by the host of said transferrin receptor protein of a strain of *Moraxella* or the fragment or the analog of the transferrin receptor protein.

12. The vector of claim 11 having the characteristics of plasmid pLEM-29, pLEM-33, pLEM-37, SLRD35-A and SLRD35-B.

13. A transformed host containing an expression vector as claimed in claim 11.

14. A method of forming a substantially pure recombinant transferrin receptor protein of a strain of *Moraxella*, which comprises:

growing the transformed host of claim 13 to express a transferrin receptor protein as inclusion bodies,

purifying the inclusion bodies free from cellular material and soluble proteins,

solubilizing transferrin receptor protein from the purified inclusion bodies, and

purifying the transferrin receptor protein free

from other solubilized materials.

15. The method of claim 14 wherein said transferrin receptor protein comprises Tbp1 alone, Tbp2 alone or a mixture of Tbp1 and Tbp2.

16. The method of claim 15 wherein said transferrin receptor protein is at least about 70% pure.

17. The method of claim 16 wherein said transferrin receptor protein is at least about 90% pure.

18. A recombinant transferrin receptor protein or fragment or analog thereof producible by the transformed host of claim 12.

19. The protein of claim 18 which is transferrin receptor binding protein 1 (Tbp1) of the *Moraxella* strain devoid of other proteins of the *Moraxella* strain.

20. The protein of claim 18 which is transferrin receptor binding protein 2 (Tbp2) of the *Moraxella* strain devoid of other proteins of the *Moraxella* strain.

21. The protein of claim 18 wherein the strain of *Moraxella* is a strain of *Moraxella catarrhalis*.

22. An immunogenic composition, comprising at least one active component selected from the group consisting of:

(A) a purified and isolated nucleic acid molecule encoding a transferrin receptor protein of a strain of *Moraxella* or a fragment or an analog of the transferrin receptor protein;

(B) a purified and isolated nucleic acid molecule having a DNA sequence selected from the group consisting of:

(a) a DNA sequence as set out in Figure 5, 6, 10, 11 or 27 (SEQ ID Nos: 1, 2, 3, 4, 5, 6, 7, 8, 45 or 46) or the complementary DNA sequence thereto;

(b) a DNA sequence encoding an amino acid sequence as set out in Figure 5, 6, 10, 11 or 27 (SEQ ID Nos: 9, 10, 11, 12, 13, 14, 15, 16 or 47) or the complementary DNA sequence thereto; and

(c) a DNA sequence which hybridizes under stringent conditions to any one of the DNA sequences defined in (a) or (b); or

(C) a recombinant transferrin receptor protein or fragment or analog thereof producible by a transformed host containing an expression vector comprising a nucleic acid molecule as defined in (A) or (B) and expression means operatively coupled to the nucleic acid molecule for expression by the host of the recombinant transferrin receptor protein or fragment or analog thereof;

and a pharmaceutically acceptable carrier therefor, said at least one active component producing an immune response when administered to a host.

23. A method for generating an immune response in a host, comprising administering to the host an immunoeffective amount of the immunogenic composition of claim 22.

24. A method of determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising the steps of:

(a) contacting the sample with the nucleic acid molecule of claim 1 or 6 to produce duplexes comprising the nucleic acid molecule and any said nucleic acid molecule encoding the transferrin receptor protein of a strain of *Moraxella* present in the sample and specifically hybridizable therewith; and

(b) determining production of the duplexes.

25. A diagnostic kit for determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising:

- (a) the nucleic acid molecule of claim 1 or 6;
- (b) means for contacting the nucleic acid molecule with the sample to produce duplexes comprising the nucleic acid molecule and any said nucleic acid present in the sample and hybridizable with the nucleic acid molecule; and
- (c) means for determining production of the duplexes.

AMINO ACID SEQUENCES OF A CONSERVED PORTION OF
Tbp1 PROTEIN FOR CONSTRUCTION OF DEGENERATE
PRIMERS USED IN PCR AMPLIFICATION OF A PORTION
OF THE *M. cattarhalis* 4223 *tbpA* GENE.

N E V T G L G	SEQ ID NO: 17
G A I N E I E	SEQ ID NO: 18

FIG.1

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M. catarrhalis 4223 Transferrin Receptor Genes

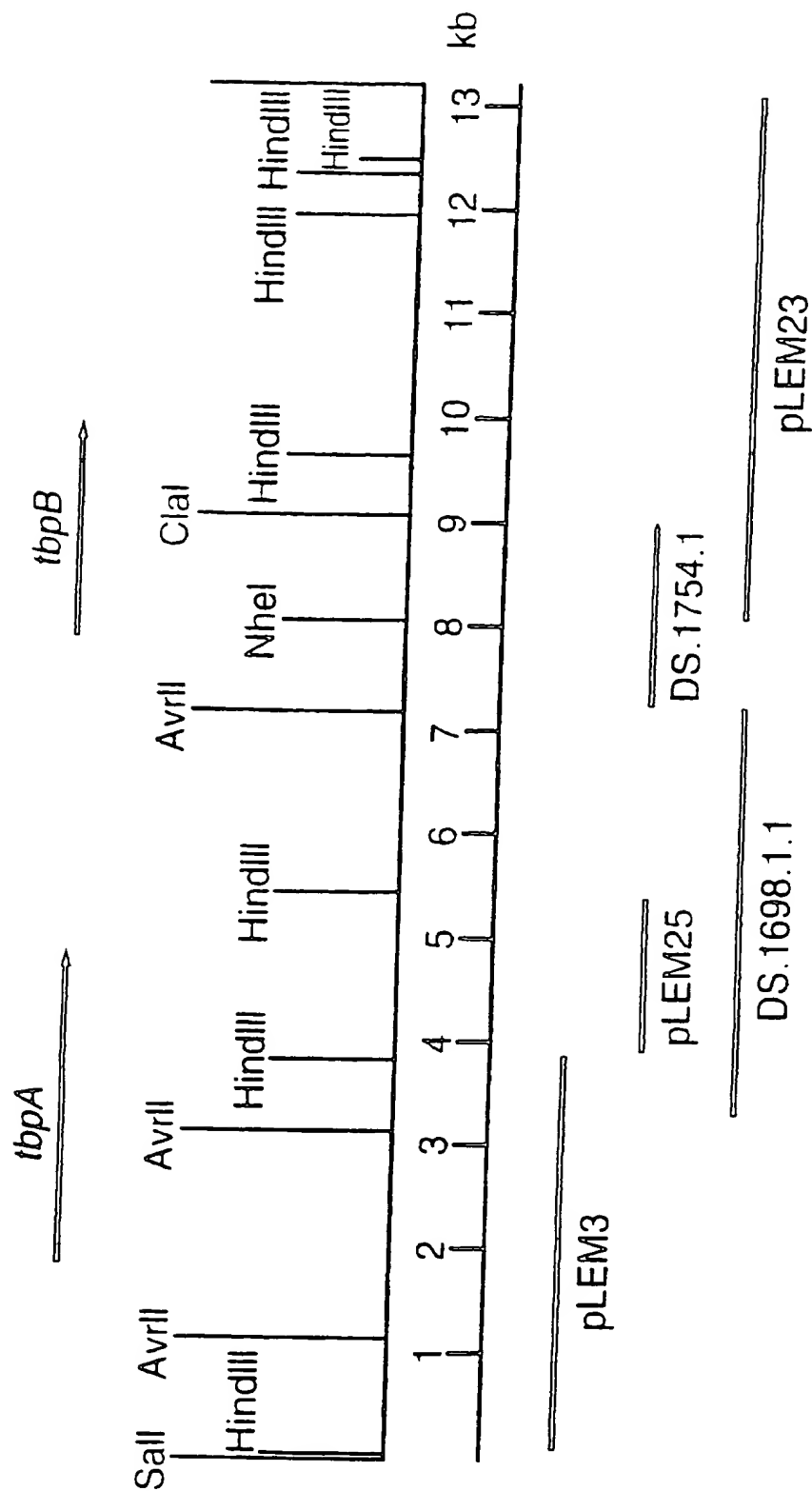


FIG.2

M. catarrhalis 4223 *tbpA* gene

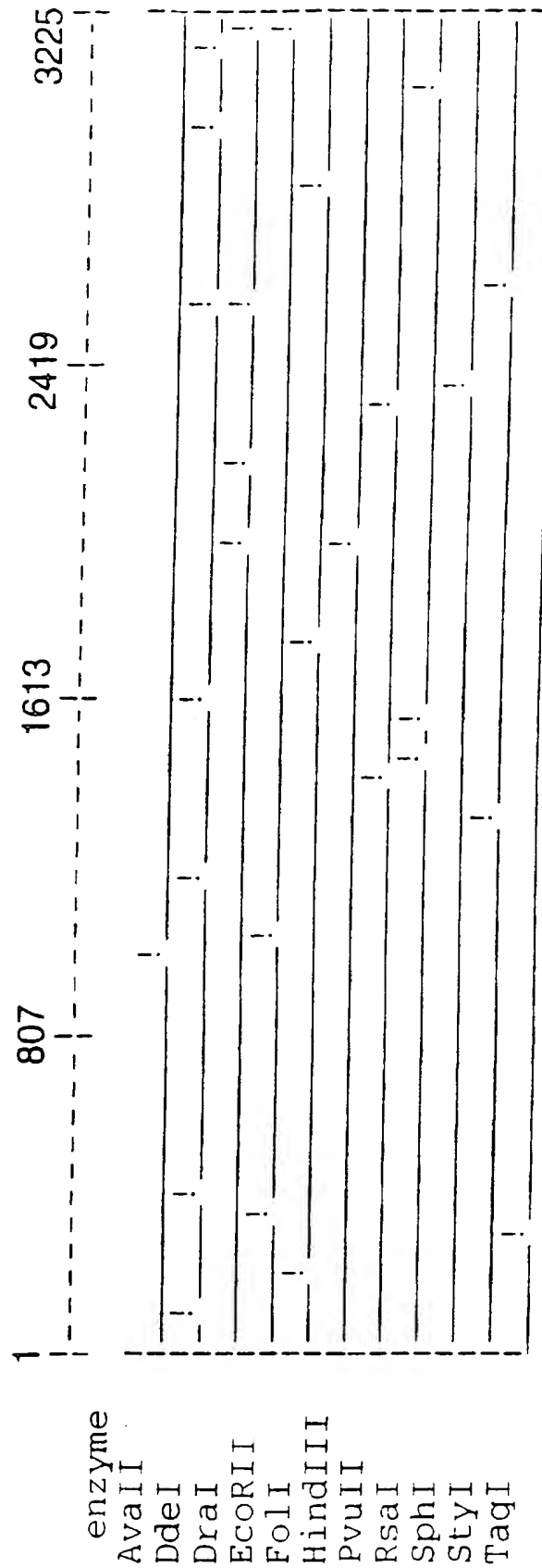


FIG.3

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M. catarrhalis 4223 *tbpB* gene

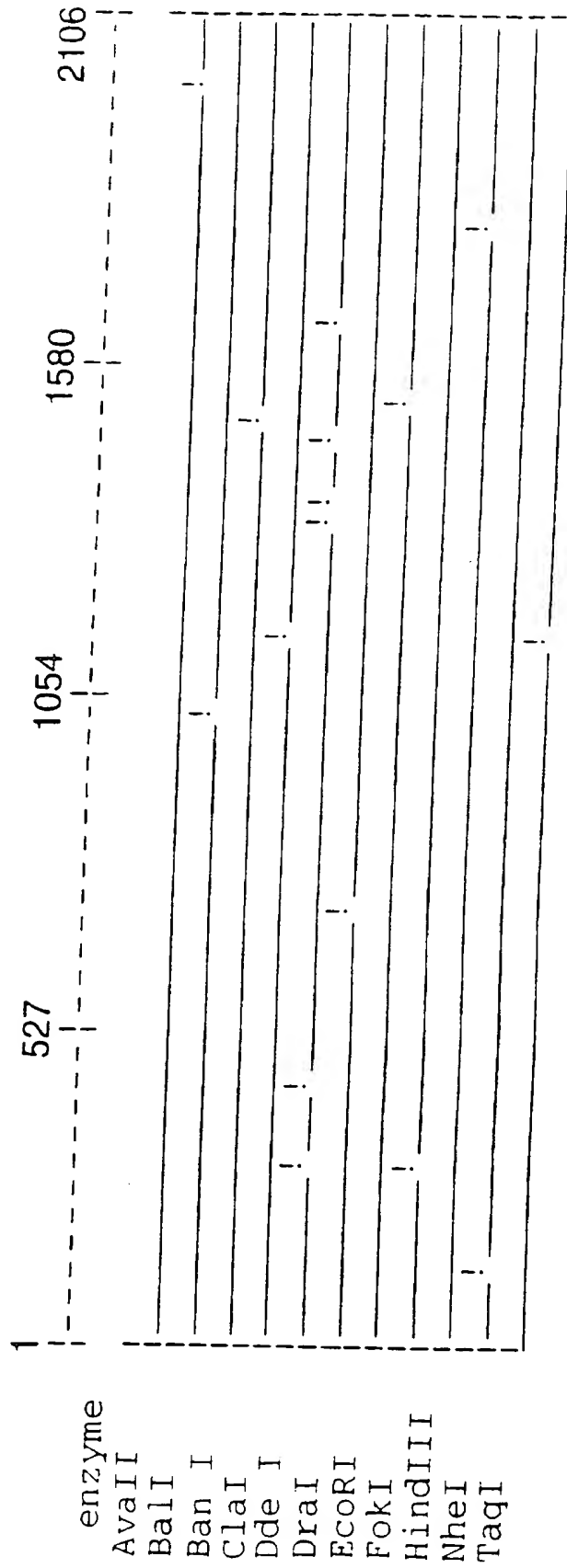


FIG.4

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FIG.5A

Sequence of *M. catarrhalis* 4223 *tbpA* gene

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TATTTTGACAAGCTATACACTAAATCAAAAATTAATCACTTTGGTTGGGTGGTTTTAGCAAGCAAATGGT
TATTTTGGTAAACAATTAAAGTTCTTAAACGATACACGCTCATAAACAGATGGTTT'TTGGCATCTGCAAT
TTGATGCCTGCCTTGTGATTGGTTGGGTGTATCGGTGTATCAAAAGTGCAAAAGCCAAACAGGTGGTCAATTG

                27
ATG AAT CAA TCA AAA CAA AAC AAC AAA TCC AAA AAA TCC AAA CAA GTA TTA AAA 54
MET Asn Gln Ser Lys Lys Gln Asn Lys Ser Lys Ser Lys Lys Gln Val Leu Lys Lys
CTT AGT GCC TTG TCT TTG GGT CTG CTG CTT AAC ATC ACG CAG GTG GCA CTG GCA AAC 108
Leu Ser Ala Leu Ser Leu Gly Leu Leu Asn Ile Thr Gln Val Ala Leu Ala Asn
                81
ACA ACG GCC GAT AAG GCG GAG GCA ACA GAT AAG ACA AAC CTT GTT GTT GTC TTG 162
Thr Thr Ala Asp Lys Lys Ala Glu Ala Thr Asp Lys Thr Asn Leu Val Val Leu
                135
GAT GAA ACT GTT GTA ACA GCG AAG AAA AAC GCC CGT AAA GCC AAC GAA GTT ACA 216
Asp Glu Thr Val Val Thr Ala Lys Lys Asn Ala Arg Lys Ala Asn Glu Val Thr
                189

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FIG.5B

GGG CTT	GGT AAG	GTG GTC	AAA ACT	GCC	GAG ACC	ATC AAT	AAA GAA	CAA GTG	CTA	270
Gly Leu	Gly Lys	Val Val	Lys Thr	Ala Thr	Glu Thr	Ile Ile	Lys Glu	Gln Val	Leu	
AAC ATT	CGA GAC	TTA ACA	CGC TAT	GAC	CCT GGC	ATT GCT	GTT GAG	CAA GGT		324
Asn Ile	Arg Asp	Leu Thr	Arg Tyr	Asp Thr	Pro Gly	Ile Ala	Val Val	Gln Gly		
CGT GGG	GCA AGC	TCA GGC	TAT TCT	ATT	CGT GGT	ATG GAT	AAA AAT	CGT GTG	GCG	378
Arg Gly	Ala Ser	Ser Ser	Tyr Ser	Ile Ile	Arg Arg	MET Asp	Lys Asn	Val Val	Ala	
GTA TTG	GTT GAT	GGC ATC	AAT CAA	GCC	CAG CAC	TAT GCC	CTA CAA	GGC CCT	GTG	432
Val Leu	Val Val	Gly Ile	Asn Gln	Ala Ala	Gln His	Tyr Ala	Leu Gln	Gly Pro	Val	
GCA GGC	AAA AAT	TAT GCC	GCA GGT	GGG	GCA ATC	AAC GAA	ATA GAA	TAC GAA	AAT	486
Ala Gly	Lys Asn	Tyr Ala	Ala Gly	Gly Gly	Ala Ile	Asn Glu	Ile Glu	Tyr Glu	Asn	
GTC CGC	TCC GTT	GAG ATT	AGT AAA	GGT	GCA AAT	TCA AGT	GAA TAC	GGC TCT	GGG	540
Val Arg	Ser Val	Glu Ile	Ser Lys	Gly Gly	Ala Asn	Ser Ser	Glu Tyr	Gly Ser	Gly	

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FIG.5C

GCA TTA TCT GGC TCT GTG GCA TTTT GTT ACC AAA ACC GCC GAT GAC ATC ATC AAA	567	594
Ala Leu Ser Gly Ser Val Ala Phe Val Thr Lys Thr Ala Asp Ile Ile Lys		
GAT GGT AAA GAT TGG GGC GTG CAG ACC ACC AAA ACC GCC TAT GCC AGT AAA AAT AAC	621	648
Asp Gly Lys Asp Trp Gly Val Gln Thr Lys Thr Ala Tyr Ala Ser Lys Asn Asn		
GCA TGG GTT AAT TCT GTG GCA GCA GCA GGC AAG GCA GGT TCT TTT AGC GGT CTT	675	702
Ala Trp Val Asn Ser Val Ala Ala Ala Gly Lys Ala Gly Ser Phe Ser Gly Leu		
ATC ATC TAC ACC GAC CGC CGT GGT CAA CAA GAA TAC AAG GCA CAT GAT GAT GCC TAT	729	756
Ile Ile Tyr Thr Asp Arg Arg Gly Gln Gln Glu Tyr Lys Ala His Asp Asp Ala Tyr		
CAG GGT AGC CAA AGT TTT GAT AGA GCG GTG GCA ACC ACT GAC CCA AAT AAC CGA	783	810
Gln Gly Ser Gln Ser Phe Asp Arg Ala Val Ala Thr Thr Asp Pro Asn Asn Arg		
ACA TTT TTA ATA GCA AAT GAA TGT GCC AAT GGT AAT TAT GAG GCG TGT GCT GCT	837	864
Thr Phe Leu Ile Ala Asn Glu Cys Ala Ala Asn Gly Asn Tyr Glu Ala Cys Ala Ala		
GGC GGT CAA ACC AAA CTT CAA GCC AAG CCA ACC AAT AAT GTG CGT GAT AAG GTC AAT	891	918
Gly Gly Gln Thr Lys Leu Gln Ala Lys Pro Thr Asn Val Arg Asp Lys Val Asn		

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FIG.5D

GTC AAA GAT TAT ACA GGT CCT AAC CGC CTT ATC CCA AAC CCA CTC ACC CAA GAC	945	972
Val Lys Asp Tyr Thr Gly Pro Asn Arg Leu Ile Pro Asn Pro Leu Thr Gln Asp		
AGC AAA TCC TTA CTG CTT CGC CCA GGT TAT CAG CTA AAC GAT AAG CAC TAT GTC	999	1026
Ser Lys Ser Leu Leu Leu Arg Pro Gly Tyr Gln Leu Asn Asp Lys His Tyr Val		
GGT GGT GTG TAT GAA ATC ACC AAA CAA AAC TAC GCC ATG CAA GAT AAA ACC GTG	1053	1080
Gly Gly Val Tyr Glu Ile Thr Lys Gln Asn Tyr Ala MET Gln Asp Lys Thr Val		
CCT GCT TAT CTG ACG GTT CAT GAC ATT GAA AAA TCA AGG CTC AGC AAC CAT GCC	1107	1134
Pro Ala Tyr Leu Thr Val His Asp Ile Glu Lys Ser Arg Leu Ser Asn His Ala		
CAA GCC AAT GGC TAT TAT CAA GGC AAT AAT CTT GGT GAA CGC ATT CGT GAT ACC	1161	1188
Gln Ala Asn Gly Tyr Tyr Gln Gly Asn Asn Leu Gly Glu Arg Ile Arg Asp Thr		
ATT GGG CCA GAT TCA GGT TAT GGC ATC AAC TAT GCT CAT GGC GTA TTT TAT GAT	1215	1242
Ile Gly Pro Asp Ser Gly Tyr Gly Ile Asn Tyr Ala His Gly Val Phe Tyr Asp		

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FIG.5E

GAA AAA CAC CAA AAA GAC CGC CTA GGG CTT GAA TAT GTT TAT GAC AGC AAA GGT Glu Lys His Gln Lys Asp Arg Leu Gly Leu Glu Tyr Val Tyr Asp Ser Lys Gly	1269	1296
GAA AAT AAA TGG TTT GAT GAT GAT GTG CGT GTG TCT TAT GAT AAG CAA GAC ATT ACG Glu Asn Lys Trp Phe Asp Asp Val Arg Val Ser Tyr Asp Lys Gln Asp Ile Thr	1323	1350
CTA CGC AGC CAG CTG ACC AAC ACG CAC TGT TCA ACC TAT CCG CAC ATT GAC AAA Leu Arg Ser Gln Leu Thr Asn Thr His Cys Ser Thr Tyr Pro His Ile Asp Lys	1377	1404
AAT TGT ACG CCT GAT GTC AAT AAA CCT TTT TCG GTA AAA GAG GTG GAT AAC AAT Asn Cys Thr Pro Asp Val Asn Lys Pro Phe Ser Val Lys Glu Val Asp Asn Asn	1431	1458
GCC TAC AAA GAA CAG CAC AAT TTA ATC AAA GCC GTC TTT AAC AAA AAA ATG GCG Ala Tyr Lys Glu Gln His Asn Leu Ile Lys Ala Val Phe Asn Lys Lys MET Ala	1485	1512
TTG GGC AGT ACG CAT CAT CAC ATC AAC CTG CAA GTT GGC TAT GAT AAA TTC AAT Leu Gly Ser Thr His His His Ile Asn Leu Gln Val Gly Tyr Asp Lys Phe Asn	1539	1566
TCA AGC CTG AGC CGT GAA GAT TAT CGT TTG GCA ACC CAT CAG TCT TAT CAA AAA Ser Ser Leu Ser Arg Glu Asp Tyr Arg Leu Ala Thr His Gln Ser Tyr Gln Lys	1593	1620

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FIG.5F

CTT GAT TAC ACC CCA CCA AGT AAC CCT	TTG CCA GAT AAG TTT AAG CCC	1647	1674
Leu Asp Tyr Thr Pro Pro Ser Asn Pro	Leu Pro Asp Lys Phe Lys Pro		ATT TTA
GGT TCA AAC AAC AAA CCC ATT TGC CTT	GAT GCT TAT GGT TAT GGT CAT	1701	1728
Gly Ser Asn Asn Lys Lys Pro Ile Cys Leu	Asp Ala Tyr Gly Tyr Gly His Asp		CAT His
CCA CAG GCT TGT AAC GCC AAA AAC AGC	ACT TAT CAA AAT TTT GCC ATC	1755	1782
Pro Gln Ala Cys Asn Ala Lys Asn Ser	Thr Tyr Gln Asn Phe Ala Ile		AAA Lys
GGC ATA GAG CAA TAC AAC CAA AAA ACC	AAT ACC GAT AAG ATT GAT TAT	1809	1836
Gly Ile Glu Gln Tyr Asn Gln Lys Thr	Asn Thr Asp Lys Ile Asp Tyr		CAA Gln
ATC ATT GAC CAA TAT GAT AAA CAA AAC	CCC AAC AGC ACC CTA AAA CCC	1863	1890
Ile Ile Asp Gln Tyr Asp Lys Gln Asn	Pro Asn Ser Thr Leu Lys Pro		TTT Phe
AAA ATC AAA CAA AGT TTG GGG CAA GAA	AAA TAC AAC AAG ATA GAC GAA	1917	1944
Lys Ile Lys Gln Ser Leu Glu Gln Glu	Lys Tyr Asn Lys Ile Asp Glu		CTT Leu
			Gly

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FIG.5G

TTT AAA GCT TAT TAT AAA GAT TTA CGC AAC	1971	GAA TGG GCG GGT TGG ACT AAT	1998
Phe Lys Ala Tyr Lys Asp Leu Arg Asn		Glu Trp Ala Gly Trp Thr Asn	Asn
AGC CAA CAA AAT GCC AAT AAA GGC ACG	2025	GAT AAT ATC TAT CAG CCA AAT	2052
Ser Gln Gln Asn Ala Asn Lys Gly Thr		Asp Asn Ile Tyr Gln Pro Asn	Gln Ala
ACT GTG GTC AAA GAT GAC AAA TGT AAA	2079	TAT AGC GAG ACC AAC AGC TAT	2106
Thr Val Val Lys Asp Asp Lys Cys Lys		Tyr Ser Glu Thr Asn Ser Tyr	Ala Asp
TGC TCA ACC ACT CGC CAC ATC ATC AGT GGT	2133	GAT AAT TAT TTC ATC GCT TTA	2160
Cys Ser Thr Thr Arg His Ile Ser Gly		Asp Asn Tyr Phe Ile Ala Leu	Lys Asp
AAC ATG ACC ATC AAT AAA TAT GTT GAT	2187	TTG GGG CTG GGT GCT CGC TAT	2214
Asn MET Thr Ile Asn Lys Tyr Val Asp		Leu Gly Leu Gly Ala Arg Tyr	Asp Arg
ATC AAA CAC AAA TCT GAT GTG CCT TTG	2241	GTA GAC AAC AGT GCC AGC AAC	2268
Ile Lys His Lys Ser Asp Val Pro Leu		Val Asp Asn Ser Ala Ser Asn	Gln Leu

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FIG.5H

TCT	TGG	AAT	TTT	GGC	GTG	GTC	GTC	AAG	CCC	ACC	AAT	TGG	CTG	GAC	ATC	GCT	TAT	2322
Ser	Trp	Asn	Phe	Gly	Val	Val	Val	Lys	Pro	Thr	Asn	Trp	Leu	Asp	Ile	Ala	Tyr	
AGA	AGC	TCG	CAA	GGC	TTT	CGC	ATG	CCA	AGT	TTT	TCT	GAA	ATG	TAT	GGC	GAA	CGC	2376
Arg	Ser	Ser	Gln	Gly	Phe	Arg	MET	Pro	Ser	Phe	Ser	Glu	MET	Tyr	Gly	Glu	Arg	
TTT	GGC	GTA	ACC	ATC	GGT	AAA	GGC	ACG	CAA	CAT	GGC	TGT	AAG	GGT	CTT	TAT	TAC	2430
Phe	Gly	Val	Thr	Ile	Gly	Lys	Gly	Thr	Gln	His	Gly	Cys	Lys	Gly	Leu	Tyr	Tyr	
ATT	TGT	CAG	CAG	ACT	GTC	CAT	CAA	ACC	AAG	CTA	AAA	CCT	GAA	AAA	TCC	TTT	AAC	2484
Ile	Cys	Gln	Gln	Thr	Val	His	Gln	Thr	Lys	Leu	Lys	Pro	Glu	Lys	Ser	Phe	Asn	
CAA	GAA	ATC	GGA	GCG	ACT	TTA	CAT	AAC	CAC	TTA	GGC	AGT	CTT	GAG	GTT	AGT	TAT	2538
Gln	Glu	Ile	Gly	Ala	Thr	Leu	His	Asn	His	Leu	Gly	Ser	Leu	Glu	Val	Ser	Tyr	
TTT	AAA	AAT	CGC	TAT	ACC	GAT	TTG	ATT	GTT	GGT	AAA	AGT	GAA	GAG	ATT	AGA	ACC	2592
Phe	Lys	Asn	Arg	Tyr	Thr	Asp	Leu	Ile	Val	Gly	Lys	Ser	Glu	Glu	Ile	Arg	Thr	
CTA	ACC	CAA	GGT	GAT	AAT	GCA	GGC	AAA	CAG	CGT	GGT	AAA	GGT	GAT	TTG	GGC	TTT	2646
Leu	Thr	Gln	Gly	Asp	Asn	Ala	Gly	Lys	Gln	Arg	Gly	Lys	Gly	Asp	Leu	Gly	Phe	

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FIG.5I

CAT AAT GGA CAA GAT GCT GAT TTG ACA GGC ATT AAC ATT CTT GGC AGA CTT GAC	2673	2700
His Asn Gly Gln Asp Ala Asp Leu Thr Gly Ile Asn Ile Leu Gly Arg Leu Asp		
CTA AAC GCT GTC AAT AGT CGC CTT CCC TAT GGA TTA TAC TCA ACA CTTG GCT TAT	2727	2754
Leu Asn Ala Val Asn Ser Arg Leu Pro Tyr Gly Leu Tyr Ser Thr Leu Ala Tyr		
AAC AAA GTT GAT GTT AAA GGA AAA ACC TTA AAC CCA ACT TTG GCA GGA ACA AAC	2781	2808
Asn Lys Val Asp Val Lys Gly Lys Thr Leu Asn Pro Thr Leu Ala Gly Thr Asn		
ATA CTG TTT GAT GCC ATC CAG CCA TCT CGT TAT GTG GTG GGG CTT GGC TAT GAT	2835	2862
Ile Leu Phe Asp Ala Ile Gln Pro Ser Arg Tyr Val Val Gly Leu Gly Tyr Asp		
GCC CCA AGC CAA AAA TGG GGA GCA AAC GCC ATA TTT ACC CAT TCT GAT GCC AAA	2889	2916
Ala Pro Ser Gln Lys Trp Gly Ala Asn Ala Ile Phe Thr His Ser Asp Ala Lys		
AAT CCA AGC GAG CTT TTG GCA GAT AAG AAC TTA GGT AAT GGC AAC ATT CAA ACA	2943	2970
Asn Pro Ser Glu Leu Leu Ala Asp Lys Asn Leu Gly Asn Ile Gln Thr		

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FIG.5J

AAA CAA GCC ACC AAA GCA AAA TCC ACC CCG TGG CAA ACA CTT GAT TTG TCA GGT	2997	3024
Lys Gln Ala Thr Lys Ala Lys Ser Thr Pro Trp Gln Thr Leu Asp Leu Ser Gly		
TAT GTA AAC ATA AAA GAT AAT TTT ACC TTG CGT GCT GGC GTG TAC AAT GTA TTT	3051	3078
Tyr Val Asn Ile Lys Asp Asn Phe Thr Leu Arg Ala Gly Val Tyr Asn Val Phe		
AAT ACC TAT TAC ACC ACT TGG GAG GCT TTA CGC CAA ACA GCA GAA GGG GCG GTC	3105	3132
Asn Thr Tyr Tyr Thr Thr Trp Glu Ala Leu Arg Gln Thr Ala Glu Gly Ala Val		
AAT CAG CAT ACA GGA CTG AGC CAA GAT AAG CAT TAT GGT CGC TAT GCC GCT CCT	3159	3186
Asn Gln His Thr Gly Leu Ser Gln Asp Lys His Tyr Gly Arg Tyr Ala Ala Pro		
GGA CGC AAT TAC CAA TTG GCA CTT GAA ATG AAG TTT TAA	3213	
Gly Arg Asn Tyr Gln Leu Ala Leu Glu MET Lys Phe .		

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FIG.6A

Sequence of *M. catarrhalis* 4223 *tbpB* gene

GTAAATTGCGGTATTTTGTCTATCATAAATGCAATTATCAAAATGCTCAAAATAACGCCAAATGCACAT

TGTCAGCATGCCAAATAGGCATCAACAGACTTTTTTTAGATAATACCATCAACCCATCAGAGGATTATTTT

ATG	AAA	CAC	ATT	CCT	TTA	ACC	ACA	CTG	TGT	GTG	GCA	ATC	TCT	GCC	GTC	TTA	TTA	54
MET	Lys	His	Ile	Pro	Leu	Thr	Thr	Leu	Cys	Val	Ala	Ile	Ser	Ala	Val	Leu	Leu	

ACC	GCT	TGT	GGT	GGC	AGT	GGT	GGT	TCA	AAT	CCA	CCT	GCT	CCT	ACG	CCC	ATT	CCA	108
Thr	Ala	Cys	Gly	Gly	Ser	Gly	Gly	Ser	Asn	Pro	Pro	Ala	Pro	Thr	Pro	Ile	Pro	

AAT	GCT	AGC	GGT	TCA	GGT	AAT	ACT	GGT	AAT	GCT	GGC	GGT	GGT	ACT	GAT	Thr	Asp	162
Asn	Ala	Ser	Gly	Ser	Gly	Asn	Thr	Gly	Asn	Ala	Gly	Gly	Gly	Thr	Asp			

AAT	ACA	GCC	AAT	GCA	GGT	AAT	ACA	GGC	GGT	ACA	AAC	TCT	GGT	ACA	GGC	AGT	GCC	216
Asn	Thr	Ala	Asn	Ala	Gly	Asn	Thr	Gly	Gly	Thr	Asn	Ser	Gly	Thr	Gly	Ser	Ala	

AAC	ACA	CCA	GAG	CCA	AAA	TAT	CAA	GAT	GTA	CCA	ACT	GAG	AAA	AAT	GAA	AAA	GAT	270
Asn	Thr	Pro	Glu	Pro	Lys	Tyr	Gln	Asp	Val	Pro	Thr	Glu	Lys	Asn	Glu	Lys	Asp	

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FIG.6B

AAA GTT TCA TCC ATT CAA GAA CCT GCC	297	ATG GGT TAT GGC ATG GCT TTG AGT AAA	324
Lys Val Ser Ser Ile Gln Glu Pro Ala		<u>MET Ala Leu Ser Lys</u>	
ATT AAT CTA CAC AAC CGA CAA GAC ACG	351	CCA TTA GAT GAA AAA AAT ATC ATT ACC	378
<u>Ile Asn Leu His Asn Arg Gln Asp Thr</u>		<u>Lys Asn Ile Ile Thr</u>	
TTA GAC GGT AAA AAA CAA GTT GCA GAA	405	GGT AAA AAA TCG CCA TTG CCA TTT TCG	432
Leu Asp Gly Lys Lys Gln Val Ala Glu		Gly Lys Lys Ser Pro Leu Pro Phe Ser	
TTA GAT GTA GAA AAT AAA TTG CTT GAT	459	GGC TAT ATA GCA AAA ATG AAT GTA GCG	486
Leu Asp Val Glu Asn Lys Lys Leu Asp		Gly Tyr Ile Ala Lys <u>MET Asn Val Ala</u>	
GAT AAA AAT GCC ATT GGT GAC AGA ATT	513	AAG AAA GGT AAT AAA GAA ATC TCC GAT	540
<u>Asp Lys Asn Ala Ile Gly Asp Arg Ile</u>		<u>Lys Lys Gly Asn Lys Glu Ile Ser Asp</u>	
GAA GAA CTT GCC AAA CAA ATC AAA GAA	567	GCT GTG CGT AAA AGC CAT GAG TTT CAG	594
Glu Glu Leu Ala Lys Lys Gln Ile Lys Glu		Ala Val Arg Lys Ser His Glu Phe Gln	

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CAA	GTA	TTA	TCA	CTG	GAA	AAC	AAA	ATT	TTT	CAT	TCA	AAT	GAC	GGA	ACA	ACC	648
Gln	Val	Leu	Ser	Leu	Glu	Asn	Lys	Ile	Phe	His	Ser	Asn	Asp	Gly	Thr	Thr	
621																	
AAA	GCA	ACC	ACA	GAT	TTA	AAA	TAT	GTT	GAT	TAT	GGT	TAC	TAC	TTG	GCG	AAT	702
Lys	Ala	Thr	Thr	Arg	Asp	Leu	Lys	Val	Asp	Tyr	Gly	Tyr	Tyr	Leu	Ala	Asn	
675																	
GAT	GGC	AAT	TAT	CTA	ACC	GTC	ACA	GAC	AAA	CTT	TGG	AAT	TTA	GGC	CCT	GTG	756
Asp	Gly	Asn	Tyr	Leu	Thr	Val	Lys	Asp	Lys	Leu	Trp	Asn	Leu	Gly	Pro	Val	
729																	
GGT	GGT	GTG	TTT	TAT	AAT	GGC	ACA	ACG	GCC	AAA	GAG	TTG	CCC	ACA	CAA	GAT	810
Gly	Gly	Val	Phe	Tyr	Asn	Gly	Thr	Thr	Ala	Lys	Glu	Leu	Pro	Thr	Gln	Asp	
783																	
GCG	GTC	AAA	TAT	AAA	GGA	CAT	TGG	GAC	TTT	ATG	ACC	GAT	GTT	GCC	AAC	AGA	864
Ala	Val	Lys	Tyr	Lys	Gly	His	Trp	Asp	Phe	<u>MET</u>	<u>Thr</u>	<u>Asp</u>	<u>Val</u>	<u>Ala</u>	<u>Asn</u>	<u>Arg</u>	
837																	
AAC	CGA	TTT	AGC	GAA	GTG	AAA	GAA	AAC	TCT	CAA	GCA	GGC	TGG	TAT	GGA	GCA	918
<u>Asn</u>	<u>Arg</u>	<u>Phe</u>	<u>Ser</u>	<u>Glu</u>	<u>Val</u>	<u>Lys</u>	<u>Glu</u>	<u>Asn</u>	<u>Ser</u>	<u>Gln</u>	<u>Ala</u>	<u>Gly</u>	<u>Trp</u>	<u>Tyr</u>	<u>Tyr</u>	<u>Gly</u>	

FIG.6D

TCT	TCA	AAA	GAT	GAA	TAC	AAC	CGC	TTA	ACT	AAA	GAA	GAC	TCT	GCC	CCT	GAT	972	
Ser	Ser	Lys	Asp	Glu	Tyr	Asn	Arg	Leu	Thr	Lys	Glu	Asp	Ser	Ala	Pro	Asp		
GGT	CAT	AGC	GGT	GAA	TAT	GGC	CAT	AGC	AGT	GAG	TTT	ACT	GTT	AAT	TTT	AAG	GAA	1026
Gly	His	Ser	Gly	Glu	Tyr	Gly	His	Ser	Ser	Glu	Phe	Thr	Val	Asn	Phe	Lys	Glu	
AAA	AAA	TTA	ACA	GGT	AAG	CTG	TTT	AGT	AAC	CTA	CAA	GAC	CGC	CAT	AAG	GGC	AAT	1080
Lys	Lys	Leu	Thr	Gly	Lys	Leu	Phe	Ser	Asn	Leu	Gln	Asp	Arg	His	Lys	Gly	Asn	
GTT	ACA	AAA	ACC	GAA	CGC	TAT	GAC	ATC	GAT	GCC	AAT	ATC	CAC	GGC	AAC	CGC	TTT	1134
Val	Thr	Lys	Thr	Glu	Arg	Tyr	Asp	Ile	Asp	Ala	Asn	Ile	His	Gly	Asn	Arg	Phe	
CGT	GGC	AGT	GCC	ACC	GCA	AGC	AAT	AAA	AAT	GAC	ACA	AGC	AAA	CAC	CCC	TTT	ACC	1188
Arg	Gly	Ser	Ala	Thr	Ala	Ser	Asn	Lys	Asn	Asp	Thr	Ser	Lys	His	Pro	Phe	Thr	
AGT	GAT	GCC	AAC	AAT	AGG	CTA	GAA	GGT	GGT	TTT	TAT	GGG	CCA	AAA	GGC	GAG	GAG	1242
Ser	Asp	Ala	Asn	Asn	Arg	Leu	Glu	Gly	Gly	Phe	Tyr	Gly	Pro	Lys	Gly	Glu	Glu	

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CTG	GCA	GGT	AAA	TTC	TTA	ACC	AAT	GAC	AAC	AAA	CTC	TTT	GGC	GTC	TTT	GGT	GCT	1296
Leu	Ala	Gly	Lys	Phe	Leu	Thr	Asn	Asp	Asn	Lys	Leu	Phe	Gly	Val	Phe	Gly	Ala	
AAA	CGA	GAG	AGT	AAA	GCT	GAG	GAA	AAA	ACC	GAA	GCC	ATC	TTA	GAT	GCC	TAT	GCA	1350
Lys	Arg	Glu	Ser	Lys	Ala	Glu	Glu	Lys	Thr	Glu	Ala	Ile	Leu	Asp	Ala	Tyr	Ala	
CTT	GGG	ACA	TTT	AAT	ACA	AGT	AAC	GCA	ACC	ACA	TTC	ACC	CCA	TTT	ACC	GAA	AAA	1404
Leu	Gly	Thr	Phe	Asn	Thr	Ser	Asn	Ala	Thr	Thr	Phe	Thr	Pro	Phe	Thr	Glu	Lys	
CAA	CTG	GAT	AAC	TTT	GGC	AAT	GCC	AAA	AAA	TTG	GTC	TTA	GGT	TCT	ACC	GTC	ATT	1458
Gln	Leu	Asp	Asn	Phe	Gly	Asn	Ala	Lys	Lys	Leu	Val	Leu	Gly	Ser	Thr	Val	Ile	
GAT	TTG	GTG	CCT	ACT	GAT	GCC	ACC	AAA	AAT	GAA	TTC	ACC	AAA	GAC	AAG	CCA	GAG	1512
Asp	Leu	Val	Pro	Thr	Asp	Ala	Thr	Lys	Asn	Glu	Phe	Thr	Lys	Asp	Lys	Pro	Glu	
TCT	GCC	ACA	AAC	GAA	GCG	GGC	GAG	ACT	TTG	ATG	GTG	AAT	GAT	GAA	GTT	AGC	GTC	1566
Ser	Ala	Thr	Asn	Glu	Ala	Gly	Glu	Thr	Leu	<u>MET</u>	Val	Asn	Asp	Glu	Val	Ser	Val	

FIG.6F

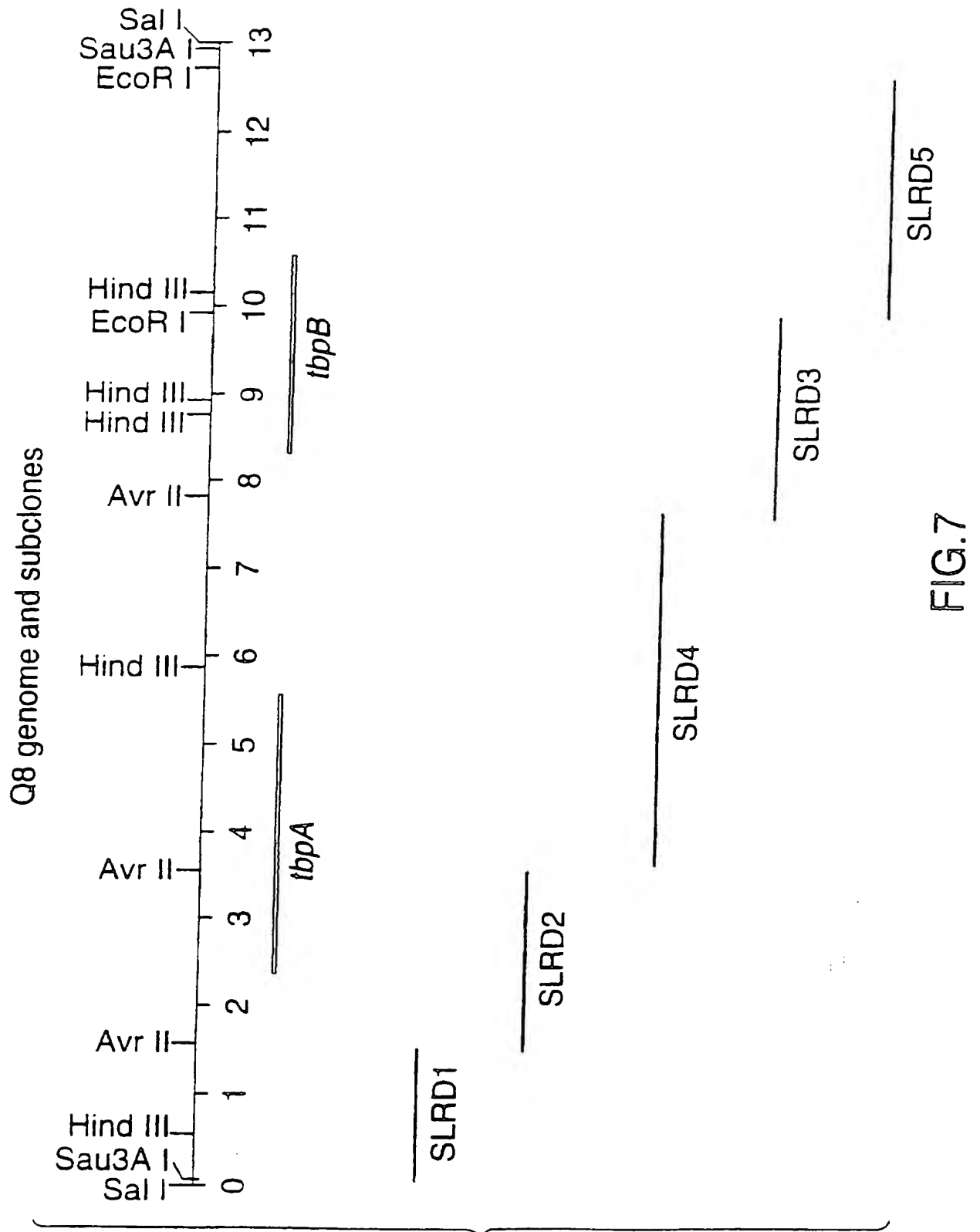
AAA ACC TAT GGC AAA AAC TTT GAA TAC CTA AAA TTT GGT GAG CTT AGT ATC GGT	1593	1620
<u>Lys Thr Tyr Gly Lys Asn Phe Glu Tyr Leu Lys Phe</u>		
GGT AGC CAT AGC GTC TTT TTA CAA GGC GAA CGC ACC GCT ACC ACA GGC GAG AAA	1647	1674
Gly Ser His Ser Val Phe Leu Gln Gly Glu Arg Thr Ala Thr Thr Gly Glu Lys		
GCC GTA CCA ACC ACA GGC ACA GGC ACA GGC AAA TAT TTG GGG AAC TGG GTA GGA TAC ATC	1701	1728
Ala Val Pro Thr Thr Gly Thr Ala Lys Tyr Leu Gly Asn Trp Val Gly Tyr Ile		
ACA GGA AAG GAC ACA GGA ACG GGC ACA GGC ACA GGA AAA AGC TTT ACC GAT GCC CAA GAT	1755	1782
Thr Gly Lys Asp Thr Gly Thr Gly Thr Gly Lys Ser Phe Thr Asp Ala Gln Asp		
GTT GCT GAT TTT GAC ATT GAT TTT GGA AAT AAA TCA GTC AGC GGT AAA CTT ATC	1809	1836
Val Ala Asp Phe Asp Ile Asp Phe Gly Asn Lys Ser Val Ser Gly Lys Leu Ile		
ACC AAA GGC CGC CAA GAC CCT GTA TTT AGC ATC ACA GGT CAA ATC GCA GGC AAT	1863	1890
Thr Lys Gly Arg Gln Asp Pro Val Phe Ser Ile Thr Gly Gln Ile Ala Gly Asn		

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FIG.6G

GGC	TGG	ACA	GGG	ACA	GCC	AGC	ACC	ACC	AAA	GCG	GAC	GCA	GGA	GGC	TAC	AAG	ATA	1944
Gly	Trp	Thr	Gly	Thr	Ala	Ser	Thr	Thr	Lys	Ala	Asp	Ala	Gly	Gly	Tyr	Lys	Ile	
																		1917
GAT	TCT	AGC	AGT	ACA	GGC	AAA	TCC	ATC	GCC	ATC	AAA	GAT	GCC	AAT	GTT	ACA	GGG	1998
Asp	Ser	Ser	Ser	Thr	Gly	Lys	Ser	Ile	Ala	Ile	Lys	Asp	Ala	Asn	Val	Thr	Gly	
																		1971
GGC	TTT	TAT	GGT	CCA	AAT	GCA	AAC	GAG	ATG	GGC	GGG	TCA	TTT	ACA	CAC	AAC	GCC	2052
Gly	Phe	Tyr	Gly	Pro	Asn	Ala	Asn	Glu	MET	Gly	Gly	Ser	Phe	Thr	His	Asn	Ala	
																		2025
GAT	GAC	AGC	AAA	GCC	TCT	GTG	GTC	TTT	GGC	ACA	AAA	AGA	CAA	CAA	GAA	GTI	AAG	2106
Asp	Asp	Ser	Lys	Ala	Ser	Val	Val	Phe	Gly	Thr	Lys	Arg	Gln	Gln	Glu	Val	Lys	
																		2079

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Chart of Q8_TBPA - Linear, length 3660

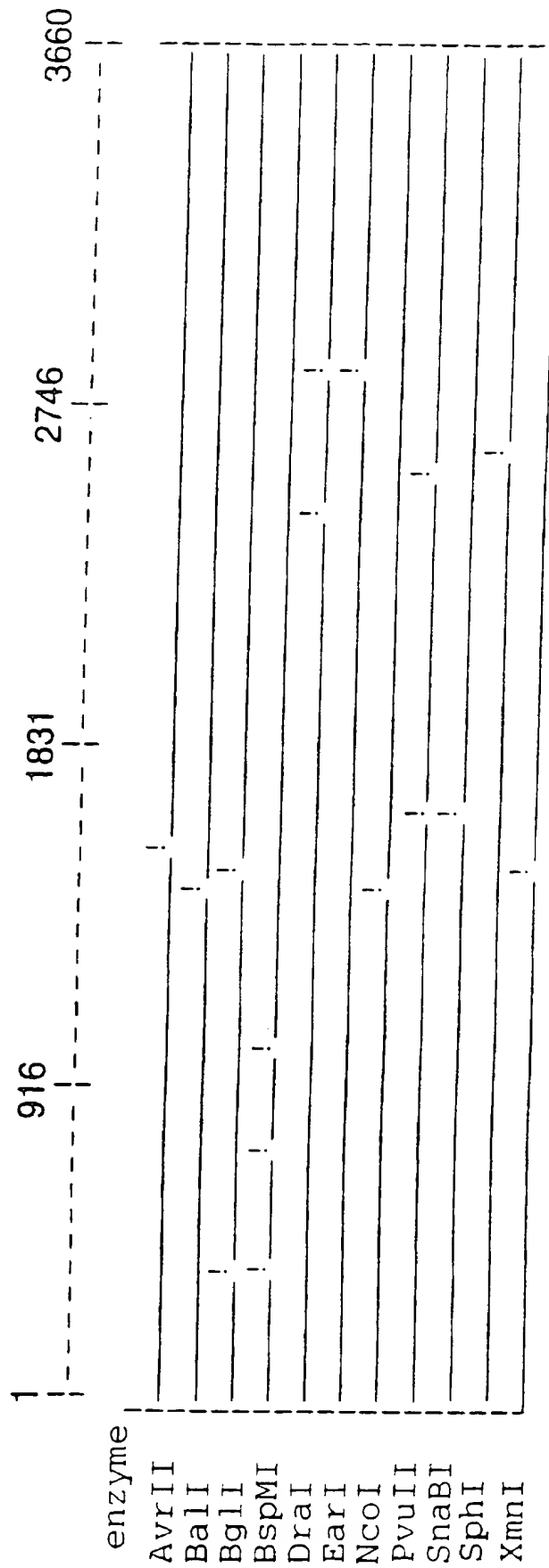


FIG.8

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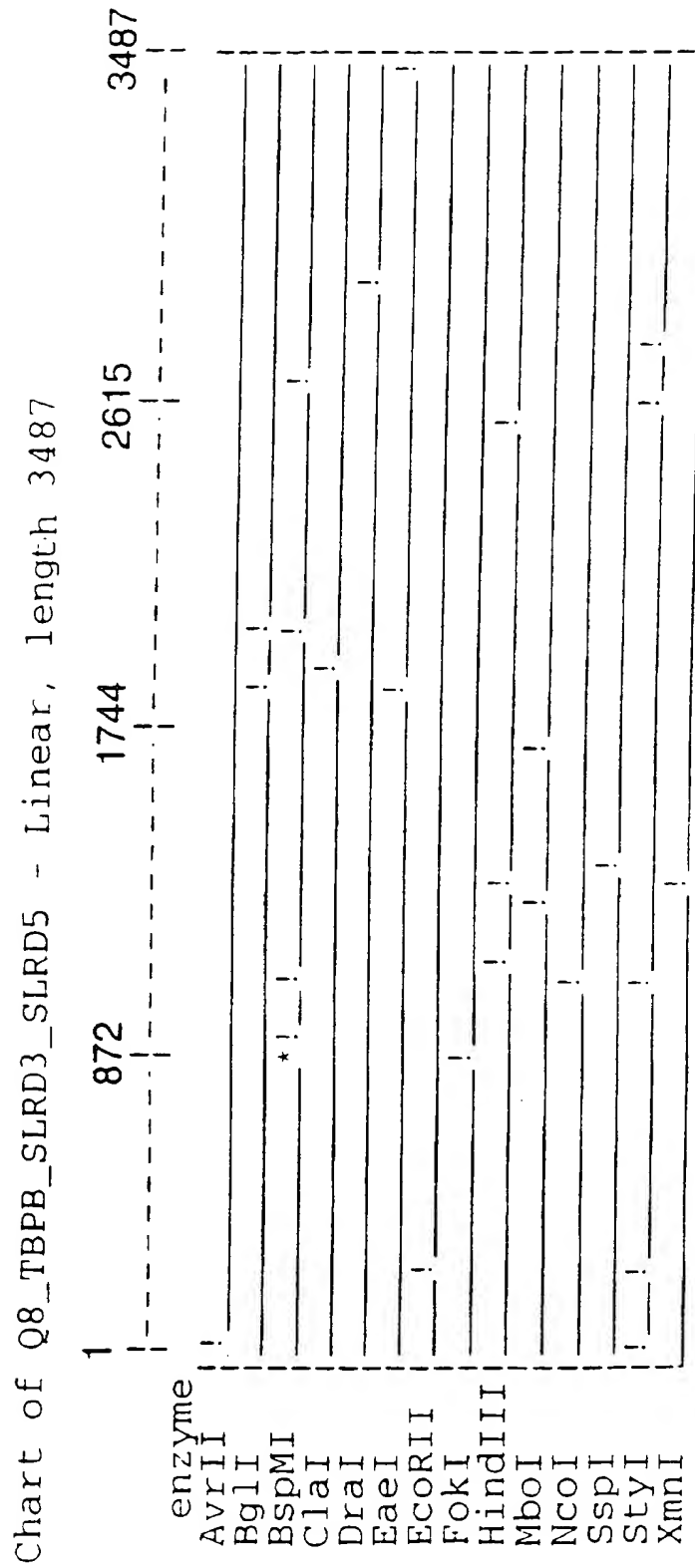


FIG.9

FIG.10A

Q8 tlpA gene sequence

A A T T G A T A C A A A T G G T T T G T A T T A T C A C T 30
10
T G T A T T G T A T T A T A A T T T A C T T A T T T T 50
40
60

A C A A C T A T A C A C T A A A A T C A A A A T T A A T 80
70
C A C T T T G G T T G G G T G G T T T A G C C A A G C A A A 110
100
120

T G G T T A T T T G G T A A C A A T T A A G T C T T A 140
130
A A A C G A T A C A C G C T C A T A A A C A G A T G G T T 170
160
180

T T T G G C A T C T T C A A T T T G A T G C C C T G C C T T G 210
190
T G A T T G G T T G G G G G T G T A T T G A T G T A T C C A 240
220
230

A G T A C A A A G C C A A C A G G T G G T C A T T G A T G 260
250
MET
270

FIG.10B

ASN GLN SER LYS LYS SER LYS SER LYS
 AATCAATCCCAAAATCCCAAAATCCCAA
 280 290 300

GLN VAL LEU LYS LEU SER ALA LEU SER LEU
 CAGTATTAAACTTAGTGCCCTTGTCCTTG
 310 320 330
 GLY LEU LEU ASN ILE THR GLN VAL ALA LEU
 GGTCCTGCTTAACATCACGCGAGTGCGACTG
 340 350 360

ALA ASN THR THR ALA ASP LYS ALA GLU ALA
 GCAACACACAGGCCGATAGGCGGAGGCA
 370 380 390
 THR ASP LYS THR ASN LEU VAL VAL VAL LEU
 ACAGATAAGACAAACCTTGTTGTGTCCTTG
 400 410 420

ASP GLU THR VAL VAL THR ALA LYS LYS ASN
 GATGAACCTGTTGTACACAGCGAAGAAAC
 430 440 450
 ALA ARG LYS ALA ASN GLU VAL THR GLY LEU
 GCCCGTAAGCCCAACGAAGTTACAGGGCTT
 460 470 480

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FIG.10C

```

GLY  LYS  VAL  VAL  LYS  THR  ALA  GLU  THR  ILE
GGTAAGGTGGTCAAAACTGCCGAGACCATC
490                                     500
                                     510
ASN  LYS  GLU  GLN  VAL  LEU  ASN  ILE  ARG  ASP
AATAAGAACAAAGTGCTAAACAATTCCGAGAC
520                                     530
                                     540

LEU  THR  ARG  TYR  ASP  PRO  GLY  ILE  ALA  VAL
TTACACGCTATGACCCCTGGCATTGCTGTG
550                                     560
                                     570
VAL  GLU  GLN  GLY  ARG  GLY  ALA  SER  SER  GLY
GTGAGCAAGGTCGCTGGGCGCAAGCTCAGGC
580                                     590
                                     600

TYR  SER  ILE  ARG  GLY  MET  ASP  LYS  ASN  ARG
TATCTATTCTGGGTATGGATAAATCGT
610                                     620
                                     630
VAL  ALA  VAL  LEU  VAL  ASP  GLY  ILE  ASN  GLN
GTGGCGGTATTGGTTGATGGCATCATCAA
640                                     650
                                     660

ALA  GLN  HIS  TYR  ALA  LEU  GLN  PRO  VAL
GCCAGCACTATGCCCTACAAAGGCCCTGTG
670                                     680
                                     690

```

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FIG.10D

ALA GLY LYS ASN TYR ALA ALA GLY GLY ALA
GCAGGCAAAATTAATGCGCAGGTGGGGCA 710 720

ILE ASN GLU ILE GLU TYR GLU ASN VAL ARG
ATCAAGAAATAGAAATACGAATAATGTCGGC 730 750
SER VAL GLU ILE SER LYS GLY ALA ASN SER
TCCGTTGAGATTAGTAAGGTGCCAAATTCA 760 780

SER GLU TYR GLY SER GLY ALA LEU SER GLY
AGTGATAACGGCTCTGGGGCAATTATCTGGC 800 810
SER VAL ALA PHE VAL THR LYS THR ALA ASP
TCTGTGGCAATTGTGTACCAAAACCGCCGAT 820 840

ASP ILE ILE LYS ASP GLY LYS ASP TRP GLY
GACATCAACAAGATGGTAAGAATTGGGGC 850 870
VAL GLN THR LYS THR ALA TYR ALA SER LYS
GTGCAGACCAAAACCGCCCTATGCCAGTAA 880 900

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FIG.10E

```

ASN  ASN  ALA  TRP  VAL  VAL  ASN  SER  VAL  ALA  ALA
A  T  A  A  C  G  C  A  T  G  G  G  T  T  A  A  T  T  C  T  G  T  G  G  C  A  G  C  A
910
ALA  GLY  LYS  ALA  GLY  SER  PHE  SER  GLY  LEU
G  C  A  G  G  C  A  A  G  G  C  A  G  G  T  T  C  T  T  T  A  G  C  G  G  T  C  T  T
940
950
960

ILE  ILE  TYR  THR  ASP  ARG  ARG  GLY  GLN  GLU
A  T  C  A  T  C  T  A  C  A  C  C  G  A  C  C  G  C  C  G  T  G  G  T  C  A  A  G  A  A
970
980
990
TYR  LYS  ALA  HIS  ASP  ASP  ALA  TYR  GLN  GLY
T  A  C  A  A  G  G  C  A  C  A  T  G  A  T  G  A  T  G  C  C  T  A  T  C  A  G  G  G  T
1000
1010
1020

SER  GLN  SER  PHE  ASP  ALA  VAL  ALA  THR
A  G  C  C  A  A  A  G  T  T  T  G  A  T  A  G  A  G  C  G  G  T  G  G  C  A  A  C  C
1030
1040
1050
THR  ASP  PRO  ASN  ASN  GLY  SER  PHE  LEU  ILE
A  C  T  G  A  C  C  C  A  A  A  T  A  A  C  C  C  A  A  A  T  T  T  T  A  A  T  A
1060
1070
1080

ALA  ASN  GLU  CYS  ALA  ASN  GLY  ASN  TYR  GLU
G  C  A  A  A  T  G  A  A  T  G  T  G  C  C  A  A  T  G  G  T  A  A  T  T  A  T  G  A  G
1090
1100
1110

```

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FIG.10F

ALA CYS ALA ALA GLY GLY GLN THR LYS LEU
 GCGTGCTGCTGGCGGTCAACCAACCTC
 1120 1130 1140

GLN ALA LYS PRO THR ASN VAL ARG ASP LYS
 CAGCTAAGCCAACTGCTGCTAG
 1150 1160 1170

VAL ASN VAL LYS ASP TYR THR GLY PRO ASN
 GTCAATGTCAAGAATTATACAGGTCCCTAAC
 1180 1190 1200

ARG LEU ILE PRO ASN PRO LEU THR GLN ASP
 CGCCTTATCCCAACCCACTCAACCAAGAC
 1210 1220 1230

SER LYS SER LEU LEU ARG PRO GLY TYR
 AGCAATCCTTACTGCTTCGCCCAAGTTAT
 1240 1250 1260

GLN LEU ASN ASP LYS HIS TYR VAL GLY GLY
 CAGCTAAGCATAGCACTATGTCGGTGGT
 1270 1280 1290

VAL TYR GLU ILE THR LYS GLN ASN THR ALA
 GTGTATGAATCACCACAAACCTACGCC
 1300 1310 1320

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FIG.10G

MET GLN ASP LYS THR VAL PRO ALA TYR LEU
 ATGCAAGATAAACCCTGCTTATCTG
 1330 1340 1350
 THR VAL HIS ASP ILE GLU LYS SER ARG LEU
 ACGGTTCAATGACATTGAAAAATCAAGGCTC
 1360 1370 1380

SER ASN HIS GLY GLN ALA ASN GLY TYR TYR
 AGCAACCATGGCCAGCCAAATGGCTATTAT
 1390 1400 1410
 GLN GLY ASN ASN LEU GLY GLU ARG ILE ARG
 CAGGCCAATAACCTTGGTGACGCAATTCGT
 1420 1430 1440

ASP ALA ILE GLY ALA ASN SER GLY TYR GLY
 GATGCCATTGGGGCAATAATTCAGGTATTGGC
 1450 1460 1470
 ILE ASN TYR ALA HIS GLY VAL PHE TYR ASP
 ATCAACTATGCTCATGGCGTATTATTATGAC
 1480 1490 1500

GLU LYS HIS GLN LYS ASP ARG LEU GLY LEU
 GAAAAACCCAAAGACCGCTAGGCTT
 1510 1520 1530

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FIG.10H

```

      GLU  TYR  VAL  TYR  ASP  SER  LYS  GLY  GLU  ASN
      G A A T A T G T T T A T G A C A G C A A A G G T C A A A T
                                     1540
                                     1550
                                     1560

      LYS  TRP  PHE  ASP  ASP  VAL  ARG  VAL  SER  TYR
      A A A T G G T T T G A T G A T G T G C G T G T C T T A T
                                     1570
                                     1580
                                     1590

      ASP  LYS  GLN  ASP  ILE  THR  LEU  ARG  SER  GLN
      G A C A G C A A G A C A T T A C G C T A C G T A G C C A G
                                     1600
                                     1610
                                     1620

      LEU  THR  ASN  THR  HIS  CYS  SER  THR  TYR  PRO
      C T G A C C A A C A C G C A C T G T T C A A C C T A T C C G
                                     1630
                                     1640
                                     1650

      HIS  ILE  ASP  LYS  ASN  CYS  THR  PRO  ASP  VAL
      C A C A T T G A C A C A A A A T T G T A C G C C T G A T G T C
                                     1660
                                     1670
                                     1680

      ASN  LYS  PRO  PHE  SER  VAL  LYS  GLU  VAL  ASP
      A A T A A A C C T T T T C G G T A A A G A G G T G G A T
                                     1690
                                     1700
                                     1710

      ASN  ASN  ALA  THR  LYS  GLU  GLN  HIS  ASN  LEU
      A A C A A T G C C T A C A A G A A C A G C A C A T T T A
                                     1720
                                     1730
                                     1740

```

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FIG.10J

```

ILE  LYS  ALA  VAL  PHE  ASN  LYS  LYS  MET  ALA
ATCAAAGCCGTCCTTAAACAATAATGGCA      1750
                                     1760
                                     1770
LEU  GLY  ASN  THR  HIS  HIS  HIS  ILE  ASN  LEU
TTGGGCAATAACGCATCATCATCAATCTG      1780
                                     1790
                                     1800

GLN  VAL  GLY  TYR  ASP  LYS  PHE  ASN  SER  SER
CAGTTGGCTATGATAATAATTCAATTCAAGC      1810
                                     1820
                                     1830
LEU  SER  ARG  ARG  GLU  ASP  TYR  ARG  LEU  ALA  THR
CTTAGCCCGTGAGATTAATCGTTTGGCAACC      1840
                                     1850
                                     1860

HIS  GLN  SER  TYR  GLN  LYS  LEU  ASP  TYR  THR
CATCAATCTTATCAAAACTTGATTACACC      1870
                                     1880
                                     1890
PRO  PRO  SER  ASN  PRO  LEU  PRO  ASP  LYS  PHE
CCACCAAGTAACCCCTTGCCAGATAAGTTT      1900
                                     1910
                                     1920

LYS  PRO  ILE  LEU  GLY  SER  ASN  ARG  PRO
AGCCCATTTTAGGTTCAACAACAGACCC      1930
                                     1940
                                     1950

```

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FIG. 10J

ILE CYS LEU ASP ALA TYR GLY HIS
 A T T G C C T T G A T G C T T A T G G T T A T G G T C A T
 1960 1970 1980

 ASP HIS PRO GLN ALA CYS ASN ALA LYS ASN
 G A C C A T C C A C A G G C T T G T A A C G C C A A A A C
 1990 2000 2010

 SER THR TYR GLN ASN PHE ALA ILE LYS LYS
 A G C A C T T A T C A A A C T T T G C C A T C A A A A A
 2020 2030 2040

 GLY ILE GLU GLN TYR ASN GLN THR ASN THR
 G G C A T A G A G C A A T A C A A C C A A T A C C
 2050 2060 2070

 ASP LYS ILE ASP TYR GLN ALA VAL ILE ASP
 G A T A A G A T T G A T T A T C A A G C C G T C A T T G A C
 2080 2090 2100

 GLN TYR ASP LYS GLN ASN PRO ASN SER THR
 C A A T A T G A T A A A C A A A C C C C A A C A G C A C C
 2110 2120 2130

 LEU LYS PRO PHE GLU LYS ILE LYS GLN SER
 C T A A A A C C C T T T G A G A A A A T C A A C A A A G T
 2140 2150 2160

FIG.10K

```

LEU  GLY  GLN  GLU  LYS  TYR  ASP  GLU  ILE  ASP
TTGGGGCCAGAGAAATACGACGAGATAGAC
2170                                2180  2190
                                ARG  LEU  GLY  PHE  ASN  ALA  TYR  LYS  ASP  LEU
                                AGACTGGGCTTTAATGCTTATAAGATTTA
2200                                2210  2220

ARG  ASN  GLU  TRP  ALA  GLY  TRP  THR  ASN  ASP
CGCAACGAATGGGCGGGTTGGACTAATGAC
2230                                2240  2250
                                ASN  SER  GLN  GLN  ASN  ALA  ASN  LYS  GLY  THR
                                AACAGCCACAACAACGCCAATAAAGGCCACG
2260                                2270  2280

ASP  ASN  ILE  TYR  GLN  PRO  ASN  GLN  ALA  THR
GATAATATCTATCAGCCAAATCAAGCAACT
2290                                2300  2310
                                VAL  VAL  LYS  ASP  ASP  LYS  CYS  LYS  THR  SER
                                GTGGTCAAGATGACAAATGTATAATAGC
2320                                2330  2340

GLU  THR  ASN  SER  TYR  ALA  ASP  CYS  SER  THR
GAGACCAACAGCTATGCTGATTGCTCAACC
2350                                2360  2370

```

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FIG.10L

THR ARG HIS ILE SER GLY ASP ASN TYR PHE
 A C T C G C C A C A T C A G C G G T G A T A T T A T T T C
 2380 2390 2400

ILE ALA LEU LYS ASP ASN MET THR ILE ASN
 A T C G C T T T A A A G A C A C A T G A C C A T C A A T
 2410 2420 2430

LYS TYR VAL ASP LEU GLY LEU GLY ALA ARG
 A A A T A T G T T G A T T T G G G G C T G G G T G C T C G C
 2440 2450 2460

TYR ASP ARG ILE LYS HIS LYS SER ASP VAL
 T A T G A C A G A A T C A A A C A C A A A T C T G A T G T G
 2470 2480 2490

PRO LEU VAL ASP ASN SER ALA SER ASN GLN
 C C T T T G G T A G A C A C A G T G C C A G C A A C C A G
 2500 2510 2520

LEU SER TRP ASN PHE GLY VAL VAL LYS
 C T G T C T T G G A A T T T T G G C G T G G T C G T C A A G
 2530 2540 2550

PRO THR ASN TRP LEU ASP ILE ALA TYR ARG
 C C C A C C A A T T G G C T G G A C A T C G C T T A T A G A
 2560 2570 2580

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FIG.10N

```

TYR  PHE  LYS  ASN  ARG  TYR  THR  ASP  LEU  ILE
T A T T T A A A A T C G C T A T A C C G A T T T G A T T
2800                                     2810 2820

VAL  GLY  LYS  SER  GLU  GLU  ILE  ARG  THR  LEU
G T T G G T A A A G T G A G A G A T T A G A C C C T A
2830                                     2840

THR  GLN  GLY  ASP  ASN  ALA  GLY  LYS  GLN  ARG
A C C C A A G G T G A T A A T G C A G G C A A C A G C G T
2860                                     2870 2880

GLY  LYS  GLY  ASP  LEU  GLY  PHE  HIS  ASN  GLY
G G T A A A G G T G A T T T G G C T T T C A T A A T G G G
2890                                     2900 2910

GLN  ASP  ALA  ASP  LEU  THR  GLY  ILE  ASN  ILE
C A G A T G C T G A T T T G A C A G G C A T T A C A C A T T
2920                                     2930 2940

LEU  GLY  ARG  LEU  ASP  LEU  ASN  ALA  VAL  ASN
C T T G G C A G A C T T G A C C T A A A C G C T G T C A A T
2950                                     2960 2970

SER  ARG  LEU  PRO  TYR  GLY  LEU  TYR  SER  THR
A G T C G C C T T C C C T A T G G A T T A C T C A C A
2980                                     2990 3000

```

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FIG.100

```

LEU  ALA  TYR  ASN  LYS  VAL  ASP  VAL  LYS  GLY
CTGGCTTATATAACAAGTTGATTAAAGGA
3010                                     3020
                                     LYS  THR  LEU  ASN  PRO  THR  LEU  ALA  GLY  THR
                                     AAAACCTTAAACCCCAACTTTGGCAGGAACA
                                     3040                                     3050                                     3060

ASN  ILE  LEU  PHE  ASP  ALA  ILE  GLN  PRO  SER
AATACTGTTTGTATGCCATTTCAGCCATCT
3070                                     3080                                     3090
                                     ARG  TYR  VAL  VAL  GLY  LEU  GLY  TYR  ASP  ALA
                                     CGTTATGTGGTGGGGCTTGGCTATGATGCC
                                     3100                                     3110                                     3120

PRO  SER  GLN  LYS  TRP  GLY  ALA  ASN  ALA  ILE
CCAGCCAAATAATGGGAGCAACGCCATA
3130                                     3140                                     3150
                                     PHE  THR  HIS  SER  ASP  ALA  LYS  ASN  PRO  SER
                                     TTACCCATTCTGATGCCAATAATCCAGC
                                     3160                                     3170                                     3180

GLU  LEU  LEU  ALA  ASP  LYS  ASN  LEU  GLY  ASN
GAGCTTTTGGCAGATAAGAACTTAGGTAAAT
3190                                     3200                                     3210

```

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FIG.10P

GLY ASN ILE GLN THR LYS GLN ALA THR LYS
 GGCACATTCACACAAACAAGCCACCAAA
 3220 3230 3240

ALA LYS SER THR PRO TRP GLN THR LEU ASP
 GCAATCCACGCCGTGGCAACACTTGAT
 3250 3260 3270

LEU SER GLY TYR VAL ASN ILE LYS ASP ASN
 TTGTCAGGTATGTAAACAATAAGATAAT
 3280 3290 3300

PHE THR LEU ARG ALA GLY VAL TYR ASN VAL
 TTACCTTGCGTGCTGGCGTGTAACAATGTA
 3310 3320 3330

PHE ASN THR TYR TYR THR THR TRP GLU ALA
 TTATAATACCTATTAACCACTTGGGAGGCT
 3340 3350 3360

LEU ARG GLN THR ALA GLU GLY ALA VAL ASN
 TTACGCCAACAAGCAGAAAGGCGGTCAT
 3370 3380 3390

GLN HIS THR GLY LEU SER GLN ASP LYS HIS
 CAGCATACAGGACTGAGCCAGATAAGCAT
 3400 3410 3420

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FIG.10Q

```

TYR  GLY  ARG  TYR  ALA  ALA  PRO  GLY  ARG  ASN
TATGGTCGCTATGCGCGCTCCCTGGACGCAAT
3430                                     3440
                                     TYR  GLN  LEU  ALA  LEU  GLU  MET  LYS  PHE  ***
                                     TACCAATTGGCCACTTGAAATGAAAGTTTAA
3480                                     S                                     3470
CCAGTGGCTTTGATGTGATCATGCCAAATC
3490                                     3500
                                     CCAATCAACCAATGAAATAAGCCCCCATCT
                                     3520                                     3530                                     3540
ACCATGAGGGCTTTATTTTATTTATCATCGCTGA
3550                                     3560                                     3570
                                     GATGCTCTTAGCGGTCATCACTCAGATT
3600                                     3580                                     3590                                     3600
GTCAATAATTATTAGCGATTAAATTATTA
3610                                     3620                                     3630
                                     GTAATCACGCTGCTCTTTGATGATTTTAAAG
3660                                     3640                                     3650                                     3660

```

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FIG.11A

Q8 tbpB Sequence.

```

CCTAGGGCTGACAGTAACAACACTTTATAC      30
      10
AGCACATCATTTGATTTATTACCCAAATGCC      60
      40
ACACGCTATTATCTTTTGGGGGCGAGACTTT      90
      70
TATGATGAATAAAGTGCCACAAGACCCATCT      120
      100
GACAGCTATGAGCGTCGTGGCATACGCACA      150
      130
GCTTGGGGGCAAGAAATGGGGCGGGCTCTT      180
      160
TCAAGCCGTGCCCCAAATCAGCATCAACAA      210
      190
CGCCATTACCAGGAGCAAAACCTAACCCAGC      240
      220
GGTGGACAAATTCCGCCAGGATAAACAGATG      270
      250
CAAGCGTCTTTATCGCTTTGGCCACAGAGAC      300
      280

```

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FIG.11B

```

ATTCAAAATGGGGCATCACGCCACGGCTG      310
                                     320
                                ACCATCAGCACAAACATCAATAAAGCAAT      350
                                     340
GACATCAAGGCAAATTATCACAAATCAA      370
                                     380
                                ATGTTTGTGTGAGTTTAGTCGCATTTTTGA      410
                                     400
TGGGATAAGCATGCCCCTACTTTTGTTTT      430
                                     440
                                GTAAAAAATGTACCATCATAGACAATATC      470
                                     460
AAGAAAAATCAAGAAAAAAGATTACAAAT      490
                                     500
                                TTAATGATAAATTGTTATTGTTTATT      530
                                     520
ATTATCAATGTAAATTTGCCGTATTTGT      550
                                     560
                                CCATCATAAACGCATTATTCAAAATGCTCAA      590
                                     600

```

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FIG.11C

```

A T A A T A C G C C A A A T G C A C A T T G T C A A C A T      610
                                     620
                                     630
G C C A A A A T A G G C A T T A C A G A C T T T T T T A G      640
                                     650
                                     660

A T A A T A C C A T C A A C C C A T C A G A G G A T T A T T      670
                                     680
                                     690
      MET  LYS  HIS  ILE  PRO  LEU  THR  THR  LEU  C
      T T A T G A A A C A C A T T C C T T T A A C C A C T G T      700
                                     710
                                     720

Y S  VAL  ALA  ILE  SER  ALA  VAL  LEU  LEU  THR
G T G T G G C A A T C T C T G C C G T C T T A T T A C C G      730
                                     740
                                     750
      ALA  CYS  GLY  GLY  SER  SER  GLY  GLY  PHE  ASN  P
      C T T G T G G T G G T A G C A G T G G T G G T T C A A T C      760
                                     770
                                     780

R O  PRO  ALA  SER  THR  PRO  ILE  PRO  ASN  ALA
C A C C T G C C T C T A C G C C C A T C C C A A A T G C A G      790
                                     800
                                     810
      GLY  ASN  SER  GLY  ASN  ALA  GLY  ASN  ALA  GLY  A
      G T A A T C A G G T A A T G C T G G C A A T G C T G G C A      820
                                     830
                                     840

```

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FIG.11D

```

SN  ALA  GLY  GLY  THR  GLY  GLY  ALA  ASN  SER
A  T G C T G G C G G T A C T G G C G G T G C A A A C T C T G
                                     860
                                     870
GLY  ALA  GLY  ASN  ALA  GLY  GLY  THR  GLY  GLY  A
G  T G C A G G T A A T G C T G G C G G T A C T G G C G G T G
                                     880
                                     890
                                     900

LA  ASN  SER  GLY  ALA  GLY  SER  ALA  SER  THR
C  A A C T C T G G T G C A G G C A G T G C C A G C A C A C
                                     910
                                     920
                                     930
PRO  GLU  PRO  LYS  TYR  LYS  ASP  VAL  PRO  THR  A
C  A G A C C C A A A A T A T A A A G A T G T G C C A A C C G
                                     940
                                     950
                                     960

SP  GLU  ASN  LYS  LYS  ALA  GLU  VAL  SER  GLY
A  T G A A A T A A A A A G C T G A A G T T T C A G G C A
                                     970
                                     980
                                     990
ILE  GLN  GLU  PRO  ALA  MET  GLY  TYR  GLY  VAL  G
T  T C A A G A A C C T G C C A T G G G T T A T G G C G T G G
                                     1000
                                     1010
                                     1020

LU  LEU  LYS  LEU  ARG  ASN  TRP  ILE  PRO  GLN
A  A T T A A G C T T C G T A A C T G G A T A C C A C A G
                                     1030
                                     1040
                                     1050

```

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FIG. 11E

GLU GLN GLU GLU GLU GLU GLU GLU GLU GLU GLU GLU GLU GLU GLU GLU GLU GLU GLU GLU
 A A C A G G A A G A A C A T G C C A A A A T C A A T A C A A
 1060 1070 1080

SN ASP VAL VAL LYS LEU GLU GLY ASP LEU
A T G A T G T T G T A A A C T T G A G G T G A C T T G A
1090 1100 1110

LYS HIS ASN PRO PHE ASP ASN SER ILE TRP G
 A G C A T A T C C A T T T G A C A A C T C T A T T G G C
 1120 1130 1140

LN ASN ILE LYS ASN SER LYS GLU VAL GLN
A A A C A T C A A A A T A G C A A G A G T A C A A A
1150 1160 1170

THR VAL TYR ASN GLN GLU LYS GLN ASN ILE G
C T G T T A C A C C A G A G A G C A A A C A T T G
1180 1190 1200

LU ASP GLN ILE LYS ARG GLU ASN LYS GLN
 A A G A T C A A A T C A A A G A G A A A A T A A C A C A C
 1210 1220 1230

ARG PRO ASP LYS LYS LEU ASP ASP VAL ALA L
GCCCTGACCAAAACCTTGATGACGTGGCAC
1240 1250 1260

FIG. 11F

```

EU  GLN  ALA  TYR  ILE  GLU  LYS  VAL  LEU  ASP
TAC  AAG  CTT  ATA  TTG  AAA  AAG  TCT  TGT  GAT  G
1270                                     1280
                                     ASP  ARG  LEU  THR  GLU  LEU  ALA  LYS  PRO  ILE  T
                                     ACC  GTC  TAC  AGA  AACT  TGC  TAA  ACC  CAT  TT
1290                                     1300                                     1310                                     1320

YR  GLU  LYS  ASN  ILE  ASN  TYR  SER  HIS  ASP
ATG  AAA  AAA  ATA  TATA  TAT  TCA  CAT  GATA
1330                                     1340                                     1350
                                     LYS  GLN  ASN  LYS  ALA  ARG  THR  ARG  ASP  LEU  L
                                     AGC  AGA  ATA  AAG  CAC  GCA  CT  CGT  GAT  TT  GA
1360                                     1370                                     1380

YS  TYR  VAL  ARG  SER  GLY  TYR  ILE  TYR  ARG
AGT  ATG  TGC  GTT  CTC  TGG  TTA  TAT  TAT  TCG  C  T
1390                                     1400                                     1410
                                     SER  GLY  TYR  SER  ASN  ILE  ILE  PRO  LYS  LYS  I
                                     CAG  GTT  ATT  CTC  TAA  TAT  CAT  CTC  CAA  AGA  AAA
1420                                     1430                                     1440

LE  ALA  LYS  THR  GLY  PHE  ASP  GLY  ALA  LEU
TAG  CTA  AAA  ACT  GGT  TTT  TGA  TGG  TGC  TTT  AT
1450                                     1460                                     1470

```

SUBSTITUTE SHEET (RULE 26)

FIG.11G

PHE TYR GLN GLY THR GIN THR ALA LYS GLN L
 TTTATCAAGGTACACAAACTGCTAAACAAT
 1480 1490 1500

EU PRO VAL SER GIN VAL LYS TYR LYS GLY
 TGCCGTGTAATCTCAAGTTAAGTATAAAGGCA
 1510 1520 1530

THR TRP ASP PHE MET THR ASP ALA LYS LYS G
 CTTGGGATTTTATGACCGATGCCAAATAAG
 1540 1550 1560

LY GLN SER PHE SER SER PHE GLY THR SER
 GACAATCATTTAGCAGTTTGGTACATCGC
 1570 1580 1590

GLN ARG LEU ALA GLY ASP ARG TYR SER ALA M
 AACGTCTTGCTGGTGATCGTTATAGTGCAA
 1600 1610 1620

ET SER TYR HIS GLU TYR PRO SER LEU LEU
 TGTCCTTACCATTGAATAACCAATCTTTATAA
 1630 1640 1650

THR ASP GLU LYS ASN LYS PRO ASP ASN TYR A
 CTGATGAGAAACAACAACCAATATAATA
 1660 1670 1680

SUBSTITUTE SHEET (RULE 26)

FIG. 11

[illegible]

FIG.11.I

```

PHE THR SER ASP ALA LYS ASN SER LEU GLU G
T T A C C A G C G A T G C C A A A A A T A G C C T A G A A G
1900 1910 1920

LY GLY PHE TYR GLY PRO ASN ALA GLU GLU
G C G G T T T T A T G G A C C A A A C G C C G A G G A G C
1930 1940 1950

LEU ALA GLY LYS PHE LEU THR ASN ASP ASN L
T G G C A G G T A A A T T C C T A C C A A T G A C A C A
1960 1970 1980

YS LEU PHE GLY VAL PHE GLY ALA LYS ARG
A A C T C T T T G G C G T C T T T G G T G C T A A A C G A G
1990 2000 2010

GLU SER GLU ALA LYS GLU LYS THR GLU ALA I
A G A G T G A A G C T A A G G A A A A A C C G A A G C C A
2020 2030 2040

LE LEU ASP ALA TYR ALA LEU GLY THR PHE
T C T T A G A T G C C T A T G C A C T T G G A C A T T T A
2050 2060 2070

ASN LYS PRO GLY THR THR THR ASN PRO ALA PHE T
A T A A C C T G G T A C G A C C A A T C C C G C C T T T A
2080 2090 2100

```

SUBSTITUTE SHEET (RULE 26)

FIG.11J

```

HR  ALA  ASN  SER  LYS  LYS  GLU  LEU  ASP  ASN
C  C  G  C  T  A  A  C  A  G  C  A  A  A  A  A  G  A  A  C  T  G  G  A  T  A  A  C  T
2110
PHE  GLY  ASN  ALA  LYS  LYS  LEU  VAL  LEU  GLY  S
T  T  G  G  C  A  A  T  G  C  C  A  A  A  A  A  G  T  T  G  G  T  C  T  T  G  G  G  T  T
2140
ER  THR  VAL  ILE  ASP  LEU  VAL  PRO  THR  GLY
C  T  A  C  C  G  T  C  A  T  T  G  A  T  T  G  G  T  G  C  C  T  A  C  C  G  G  T  G
2170
ALA  THR  LYS  ASP  VAL  ASN  GLU  PHE  LYS  GLU  L
C  C  A  C  C  A  A  A  G  A  T  G  T  C  A  A  T  G  A  A  T  T  C  A  A  A  G  A  A  A
2200
YS  PRO  LYS  SER  ALA  THR  ASN  LYS  ALA  GLY
A  G  C  C  A  A  A  G  T  C  T  G  C  C  A  C  A  A  A  C  A  A  A  G  C  G  G  C  C  G
2230
GLU  THR  LEU  MET  VAL  ASN  ASP  GLU  VAL  ILE  V
A  G  A  C  T  T  T  G  A  T  G  T  G  A  A  T  G  A  T  G  A  G  T  T  A  T  C  G
2260
AL  LYS  THR  TYR  GLY  TYR  GLY  ARG  ASN  PHE
T  C  A  A  A  C  C  T  A  T  G  G  C  T  A  T  G  G  C  A  G  A  A  A  C  T  T  T  G
2290

```

SUBSTITUTE SHEET (RULE 26)

FIG. 11K

GLU TYR LEU LYS PHE GLY GLU LEU SER ILE G
 A A T A C C T A A A A T T T G G T G A G C T T A G T A T C G
 2320 2330 2340

LY GLY SER HIS SER VAL PHE LEU GLN GLY
 G T G G T A G C C A T A G C G T C T T T T A C A A G G C G
 2350 2360 2370

GLU ARG THR ALA GLU LYS ALA VAL PRO THR G
 A A C G C A C C G C T G A G A A A G C C G T A C C A A C C G
 2380 2390 2400

LU GLY THR ALA LYS TYR LEU GLY ASN TRP
 A A G G C A C A G C C A A A T A T C T G G G A A C T G G G
 2410 2420 2430

VAL GLY TYR ILE THR GLY LYS ASP THR GLY T
 T A G G A T A C A T C A C A G G A A A G G A C A C A G G A A
 2440 2450 2460

HR SER THR GLY LYS SER PHE ASN GLU ALA
 C G A G C A C A G G A A A A G C T T T A A T G A G C C C
 2470 2480 2490

GLN ASP ILE ALA ASP PHE ASP ILE ASP PHE G
 A A G A T A T G C C T G A T T T T G A C A T T G A C T T T G
 2500 2510 2520

SUBSTITUTE SHEET (RULE 26)

FIG. 11L

```

LU  ARG  LYS  SER  VAL  LYS  GLY  LYS  LEU  THR
AGAGAAATCAGTTAAAGGCCAAACTGACCA
2530                                2540
                                THR  GLN  GLY  ARG  GLN  ASP  PRO  VAL  PHE  ASN  I
                                C C C A A G G C C G C C A A G A C C C T G T A T T T A A C A
                                2550                                2560                                2570                                2580

LE  THR  GLY  GLN  ILE  ALA  GLY  ASN  GLY  TRP
TCACAGGTCAAAATCGCAGGTAAATGGCTGGA
2590                                2600                                2610
                                THR  GLY  THR  ALA  SER  THR  ALA  LYS  ALA  ASN  V
                                CAGGCCACAGCCAGCACCGCCCAAAGCGAACCG
                                2620                                2630                                2640

AL  GLY  GLY  TYR  LYS  ILE  ASP  SER  SER  SER
TAGGGGCTACAAAGATAGATTCTAGCAGTA
2650                                2660                                2670
                                THR  GLY  LYS  SER  ILE  VAL  ILE  GLU  ASN  ALA  L
                                CAGGCCAAATCCCATCGTCAATCGAAATAATGCCA
                                2680                                2690                                2700

YS  VAL  THR  GLY  GLY  PHE  TYR  GLY  PRO  ASN
AGGTACAGGTGGCTTTTATGGTCCCAATG
2710                                2720                                2730

```

SUBSTITUTE SHEET (RULE 26)

FIG. 11M

ALA ASN GLU MET GLY GLY PHE THR HIS A
 C A A C G A G A T G G C G G G T C A T T T A C A C A C G 2760
 2740

SP THR ASP ASP SER LYS ALA SER VAL VAL
 A T A C C G A T G A C A G T A A A G C C T C T G T G G T C T 2790
 2770

PHE GLY THR LYS ARG GLN GLU VAL LYS *
 T T G G C A C A A A A G A C A A G A G A G T T A A G T 2820
 2800

**

A G T A A T T A A C A C A A T G C T T G G T T C G G C T 2850
 2830

G A T G G G A T T G A C G C T T A A T C A A A C A T G A A T 2880
 2860

G A T T A G A T G A T A A A C C C A A G C C A T G C C A A 2910
 2890

T G A T T G A T A G C A A C G A T G G C A G A T G A T G A G 2940
 2920

T T T T C A T T A T C T G C C A T T A T T A T T G C T T A A 2970
 2950

T T A T T G C T T G T C A T T T G G T G G T G T A T C A C 3000
 2980

SUBSTITUTE SHEET (RULE 26)

FIG.11N

A T T A A T C A T : A A A A T T A A C A T A A T A A A T G A 3010
 3020
 T T A A A T G A T A T T T A A T G A A A G T C A G G G T T A 3040
 3050 3060
 T T T T G G T C A T G G T T T T C A T G A T T A T T T A A 3070
 3080 3090
 C T T A T A A T G C G T T A T G G T T A G C A A A A G C T 3100
 3110 3120
 A A G T C T G T C A A T G A A G C T A T G G T G A G T G A T 3130
 3140 3150
 T G T G C A A A A G A T G G T C A A A A A A T C G G T A T 3160
 3170 3180
 G G T G C T G T C A G G C G T G G T G A T G G T T C T G T T 3190
 3200 3210
 A A T G A T A A T A A C A A C G C C A A G C C A T G C T A C 3220
 3230 3240
 T G C C A A G T T G T T G C C G A C C T C T C A A G A A A A 3250
 3260 3270
 T C C A A C C A A A A C T A T G G T A G A T A G C T T T G G 3280
 3290 3300

SUBSTITUTE SHEET (RULE 26)

FIG. 11.O

TCGTGAACGCCACGAGGGGCGAGTTTCAGGG 3310
3320
GCTATTGCCGTGCCAATTGCCAGCAGAGACTA 3340
3350
3360

TGAGCTGGCTGCCCAACTATTTTGGACGGCCG 3370
3380
TTATTGGCCAAACCCCAACGCCCAATCG 3400
3410
3420

TGAGATTGTTGAGCA 3430

SUBSTITUTE SHEET (RULE 26)

Tbpl alignment

10	20	30	40	50	60
MQSKQNKSKKQVLKSALSIGLNI--TQVALANTADKAFA-TDKTNLVVILDETIVVT- - - - - .Q.QHLFR-----NILC...-----MT..PVY-----NVQAEOAQEKQ..TIQ.K .Q.QHLFR-----NILC...-----MT..PAY-----NVQACQAEKQ..TIQ.K .Q.QHLFR-----NILC...-----MT..PAY-----NVQACQAEKQ..TIQ.K .TKKPVR-----LSIISC.LI.CYVKAE..SIKDITKE.ISS.VD.QS.E-DSE.ETIS.. AKKNA-RKANEV TGLGKVKTAE TINKEQVLNIRDLTRYDP- - - - - ...QKT.RD.....L..SSD.LS..... ...QKT.RD.....L....D.LS....D..... ...QKT.RD.....L....D.LS....D..... .E.IRD..D.....II..S.S.SR.....					
4223	Q8	B16B6	M982	FAL9	Eagan

110	120	130	140	150	160
GLAWEQFCGASSYSIRGMDKNRVAVLVDGINAQHYALQCPVAGKNYA-AGGAINETIEYEJ					
.....					
.....		SLT...	VS. I. S. TA. AALG. TRT. GSS.		
.....		SLT...	LA. I. S. TA. AALG. TRT. GSS.		
.....		SLT...	LA. I. S. TA. AALG. TRT. GSS.		
.....		R...	LP. T. S. W. S. LVATSGYSGT.		

FIG. 12B

170	180	190	200	
VRSEISKANSSEYCGSALSGSVAFVTKTADDIIKDG				4223
.....				Q8
.KA.....S.....N...A.....Q....A...GE.				B16B6
.KA.....S..V.Q.....A.....Q.....V.GE.				M982
.KA.....S..V.Q.....A.....Q.....V.GE.				FA19
.KA.....GS.....N...A...T.QS.S.A..IEGD				Eagan
210	220	230	240	250
KDWGVQTKTAYASKNNWNSVAACKAGSFSGLLIYTDRRGQEQYKAHDDAYQGSQSFDRAVA				260
.....				
.Q..I.S....SG.DH.LTQ.L.L..RS.GAEA.L...K...R.IH..K..GK.V...N.L.L				
RQ..I.S....SG..RGLTQ.I.L..RI.GAEA.L.H.G..AG.IR..E..GR.V...N.L.P				
RQ..I.S....SG..RGLTQ.I.L..RI.GAEA.L.H.G.HAG.IR..EA.GR.V...N.LAP				
.S..I...N..S....KGFTH.L.V...Q.G.E..A...Q.NSI.TQV.K..LK.V..Y..LI.				
270	280	290	300	
TTD-----PNNRTFLIANECANFNVEACAAGQTKLOAKPTN				4223
.....PK.....				Q8
DE.KKEQGSQY.Y.IVEE..H...-..A..KNKL--..ED.SVKD				B16B6
VE.-----SSEYAY.IVED..EJK...T.KSKP--..KDWGCKD				M982
VE.-----GSKYAY.IVEE..K..GH.K.K.NP--..KDWGED				FA19
...-----KSSGY.V.QG..P...DDK-.....PP.TLST				Eagan

FIG.12C

310	320	330	340	350	360	
VRDKVNVKDYTGPNRLIPNPLTQDSKSLILRPGYQLNDK-HYVGGVWEITKQNYAMQDKTVPA						
.....						
E.KT.STQ....S...LA...EYG.Q.W.F...WH.DNR-....A.L.R.Q.TFDIR.M....						
E.QT.STR.....FLAD..SYE.R.W.F...FRFENKR..I..IL.H.Q.TFDIR.M....						
K.QT.STR.....FLAD..SYE.R.W.F...FRFENKR..I..IL.R.Q.TFDIR.M....						
QSET.S.S....A..IK...MKYE.Q.WF..G..HFSEQ-.I..IF.F.Q.KFDIR.M.F...						
	370	380	390	400		
	YLTIVHDIEKSRLSNHAQA--NGYYQGNNLGERIRDTIGPD				4223	
G.---				Q8	
	.F.SE.YVPGS.KGL-----K.S.D.KA..LFVQGECS				B16B6	
	F..KAVFDANSKQAGSLPG-.K.A..HKYGGFLTNGENG				M982	
	F..KAVFDANQKQAGSLPG-.K.A..HKYGGFLTSGENN				FA19	
	..SPTERRDDSSRSFYTMQDH.A..HIE-----				Eagan	

410	420	430	440	450	460
-----SGYGINYAHGVFYDEKHQDELGLEVVYDSKGENKWFDDVRVSYDKQDITLRSQLTNITC					

TLQGI...---T.....R.T.N.Y.V...HNADKDT.A.YA.L...R.G.D.DNR.QQ....					
---ALV.AE.GT.....T.T.S.Y.....TNADKDT.A.YA.L...R.G.G.DNHFOQ....					
---APV.AE.GT.....T.T.S.Y.....TNADKDT.A.YA.L...R.G.G.DNHFOQ....					
---D.R.VK...S.LYF..H.R.Q.V.I..I.EN.NKAGII.KAVL.ANQ.N.I.D.YMRH...					

470		480	490	500	
	STYPHIDKNCPTDVKPKFVSKEVDNNAYKEQHNLIKAVFN				4223
R.G...Y.FYKS.RMI.E.SR..FQ...K				Q8
	HDGS-.....				R16B6
	ADGS-...Y.R.SAD....YYKS.RVI.G.S.R.LQ.A.K				M982
	ADGS-...Y.R.SAD....YYKS.RVI.G.S.K.LQ.A.K				FA19
	L.NPS...R.TLD.Y.YYRS.R.V...K.MLQLNLE				Eagan

510	520	530	540	550	560
KKMALGSTHHHNLQVGYDKFNSSLSREDYRLATHQSYQKLDYTPPSNPLPKF-KPILGSNN					
.....N.....					
..AFDTAKIR.NLSINL..	R.K.Q.HS.Y.QNAVQAYD.I..	KP.F.NGS..	-----D		
..SFDTAJUR.NKSVNK.F.R.S.B.RHQ..	YYQHANRAYSSK..	KTAN.NGD..	-----S		
..SFDTAKIR.NLSVNL..	T.G.N.RHQ..	YYQSANRAYS.K..	Q.NGKRTS..	PN.REK	
...IQQWLT.Q.VFNL.F.D.T.A.QHK..	-TRRVIATA-	SI.RK---	GETG..	RN.LQS	

570	580	590	600	
KPICLDAYGYGIDHPQACNAKNSTYQNFAIKKGIEQYN				4223
R.....				Q8
N.YRVSIGK----				B16B6
..YWSIG,-----				M982
N.YWWSIGR-----				FA19
Q.YLYPKPEP-----				Eagan

FIG.12E

610	620	630	640	650	660	
QKTNIDKIDYQAIIDQYDKQNPINSTILKPFEEKIKOSLGQEKYNKIDELGFKAYKDLRNEWAGMT						
.....V.....						
.....DE..R..N.....						

	670	680	690	700		
	NUNSQQVANKGRDNITYQPNQA-TVVKDDKCKYSEINS-Y					4223
					Q8
	-----T..NISPI..RFEN-.T-. .					B16B6
	-----GN..TCQI..LFEN-.T-. .					N982
	-----GN..TRQI..LFEN-.T-. .					FA19
	-----YFAGQDH-.N..QGSS.N.					Eagan
710	720	730	740	750	760	
ADCSTTRHISGDNVFIALKDNMTINKYVDLGLGARYDRIKHKSDVPLVDNSASNQLSNMFGW						
.....						
T...-P.N.G.NG.YA.VQ..VRLGRWA.V.A.I...YRSTH.EDKS.STGTHRN....A...						
T...-P.S.N.KS.YA.VR..VRLGRWA.V.A.L...YRSTH..DGS.STGTHRT....A.I.						
T...-P.S.N.KS.YA.VR..VRLGRWA.V.A.L...YRSTH..DGS.STGTHRT....A.I.						
R...-KV.L.K.K..YF.ARN..ALG.....I...VSRT.ANESTISVGKFNIF....T.I.						

FIG. 12F

	770	780	790	800	
	VKPTWLDIAYRSSQGRMPSEMYGERFGVTICKG				4223
				Q8
	L..FT.M.LT..A.T...L...A...W.A.ESLCTL				B16B6
	L..AD...LT..T.T...L...A...W.S..OSKAV				M982
	L..AD...LT..T.T...L...A...W.S.DK.KAV				FA19
	I...E...LS..L.T...N.....W.Y.GKNDV				Eagan
810	820	830	840	850	860
TQHCKGLYYICQQTIVHQTILKPEKSFNQEIGATLHNHILGSLEVSYFKNRYTDLIVGKSEIR					
.....					
-----D.....R.A.IVFKGDF.N..A...N.A.R...AFGY-.T.					
-----ID.....K.A.IVFKGDF.N..A.W.N.A.R...RGY.AQI					
-----ID.....K.A.IVFKGDF.N..A.W.N.A.R...RGY.AQI					
-----YVG.F...T.R...F.LA.KGDF.NI.I.H.S.A.RN..AFA-.LS					
	870	880	890	900	
	TLTQCDNACKQRKGDLGFHNGQDADLTGINILGRID				4223
N.....K..				Q8
	-----QN.QTSAS..P..R.A.N.RIA.....KI.				B16B6
	K-----N..EEA...PAYL.A.S.RI.....KI.				M982
	K-----D..EQV..NPAYL.A.S.RI.....KI.				FA19
	K-----NGT...NY.Y..A.N.K.V.V..TAQ..				Eagan

Top2 comparison

10	20	30	40	50	60	
MKHIPLTTL	CV AISAV - LLTACGS - GGSNPPAP	TIPI	PNASGS	ENIC	ENAGGTINT - ANAG	
.....	S . F	GN . A . A	GCANSG . .		
NN - . .	VNQAMVLP . F . S . L . G -	-----				
NN - . .	VNQAMVLP . F . S . L . G -	-----				
NN - . .	VNQAMVLP . F . S . L . G -	-----				
SV . . .	ISGGLS - - - - F . S . S . - - - -	-----				
			70	80	90	100
			NIQGT - - -	NSGIGSANTPEPKYQDV	PTKNEKDK - VSSIQEPAM	4223
			A	GA	A	Q8
			-FDLDSVE - - -	VQUMHSK	EDEKS - QP . SQOD . .	ENSGA . -
			-FDLDSVD - - -	EAPRPA -	SS . .	FQAQ . D - - - -
			-FDLDSVD - - -	EAPRPA -	PSK . P . AR . D - - - -	QG
			-FDLDSVD - - -	SK . R	DTSNQPK . S - NLKKLFI . SL	4223

110	120	130	140	150
GYGVALSKINLHNQDTPLD-EKNIITL--	DGKQVAEG-KKSPLPFS-LDV-ENKLLDGVIA			
VE.-.LRNWIP.EQEETH-A.IN-N--	VV.LEGDL--HN.FDN.IWQNIK.SKEVQTVY			
--.F.V-.LPRR.AHEN.KYK.HKP.GSM.W-----	-LQGEPSNFS.RDE.E-----			
...F.M-RLKRR.WYP--GAE.SEVK.NES.WEATGLPTKP.E-..	KRQKS.I.KVET..D-S			
...F.M-RFKRR.WHPSANPK.DEVK.KND.WEATGLPTEP.K-..	LKQOS.ISEVETN.N-S			
.G.K.VAQ..RGNKEPSFTN.DDY.-----	SY..S.STI.KDKV.NNK-			

FIG. 13B

160	170	180	190	200	
KNWADKNAIGDRIKKNKEISDEELAKQIKEAVRKSHFQQV-					4223
NQEKQNIEDQIK.EN.QRPDKLDDV.L.AYIEKVLDDRLTELA					Q8
-----K.R.SS.LI-.SKWEDQSR.VGYTN.T----					B16B6
DIYSSPYLTPSNHQNG-----AGNGVN.P.NQATGHEN.----					M982
..YTSPYLSQDADS-----HANG.N.P.NE.TDVKK.----					FA19
--G.--L..S-.-----PSTINPP.K-----HG.----					Eagan

210	220	230	240	250	260	
LSSLENKIFHSNDGTTKATTRDLKYVDYGY-YLANDGNVLTVKTKLMLGPGVGFYNGTTT						
KPIY.KN.NY.H.KQN..R.....RS..I.RSGYS.---IIPK.IAKT.FD.AL..Q..Q.						
-----RS.V.-.KN.IDIKNNIV.F--.D.YLY.K.KEP						
-----YS.WF.KH.ASEKDFSN.KI.S---DD.YI..H.EK						
-----YS.WF.KH.KSEVKNENGLVSAKR-.D.YI..H.DK						
-----YS.LY.TPSWSLND-S.N-FY-..YY.YA..Y.NK.						
	270	280	290	300		
	AKELPTQDAVKYKGHWDFMTDVANRRNRFSEVKENS--QA				4223	
	..Q..VSQ-.....T.....-KKGQS..SFGT-.QRL.				Q8	
	S.....SEKIT...T..YV..AME-KQ..-GLG-.A..G				B16B6	
	PSRQ..ASGK.I...V.H.V..TKGQD.R.IIQP.KK.G				M982	
	PSRQ..ASE..T...V.H.V..TKQQK.NDIL.T.KG.G				FA19	
	.TN..VNGVA....T...I.ATK.-GK.YPLLSNG.H.--				Eagan	

SUBSTITUTE SHEET (RULE 26)

BNSDOCID: <WO 9732980A1>

FIG.13D

450 460 470 480
 ----AILEDALGTFNTSNAT--TFTPTFEKQLNFGNAKLIV
 ----.....KPGT.NPA..ANSK.E.....
 ----TVI...RIT-----GEEFKKE.I.S.DV...L
 NSKLTIV...VE.T-----LNDKKI.N....S.AQ..
 ETRLTIV...VE.T-----PDGKEI.N....S..TR..
 KINATISPA.NTTTDTITANTI.D--EKN.KTEDISS..E.DV.L

4223
 Q8
 B16B6
 M982
 FA19
 Eagan

490 510 520
 LGSTVIDLVP-----TDAIK--NEFTKDK--PESATNEAGETLMNDEVSV--
G...DV...E...K...K.....I...
 VDGVELS.L.--SE-GNKA--FQHEI...
 VDGIM.P.L.KDSESGVIOADKGNKG--T...RKFEHT...DKKD.QAGTQINGAQIASNTA
 VDGIM.P.L.--TESGNGQADKGNKG--TD..YETTYT...DKKDTKAQTGAGGQOTASGTA
 IDKYP.P.L.-----DKNTN-----FI.SK--
 530 540

4223
 Q8
 B16B6
 M982
 FA19
 Eagan

-----KTYGKN-----FEYLFKGELSIQGS
 -----YGRN-----
 --QNGVKAT-----VCCSNLD.MS..K..KENKD
 GDTNGK--T...EVE-VCCSNLN...Y.M.TRKN.K
 GVNGGQVGT...KVQ-VCCSNLN...Y.L..RENN
 --HHTVGN-.R.KVEAVCCSNLSDVKS.MYVEDPLKE

FIG.13E

550 560 570
-----SVFLQGERTATIGEKAVPTTGTAKYLG
-----E.....

DM...V..PVSDVA.-R.EAN...R.
-----NSSQADAKTEQVEQ.M.....D..EI..DQWV.R.
SVMQAVK-----NSSQADAKTKQIEQ.M.....D.NKI.QEQGIV....
KETETETETIEKKEKEKDKKEKQTAATINITYYQ--..L.H..---PKDDI.K.S...H.

580 590 600 610
NMWGYIT-CKDTGTGTCKSFIDAQDVADFTIDFCKSVSGK
.....S.....NE...I...D...ER...K...
T.Y...AN.-TSWS.EA-.NQECCNR.E.DV..ST.KI..T
S.Y.H.AN.-TSWS.NA-.DKECCNR.E..VN.AD.KIT..
F.Y.R.AN.-TSWS.KA-.NATDCNR.K..VN.DR.EIT.T
S.Y....D..TSYSPS.DKKR.KNA..E.NV..AE.KLT.E

4223
Q8
B16B6
M982
FA19
Eagan

620 630 640 650 660 670
LITKGRQDPVFSITGQIAG--NGWIGTASTIKADAGYKIDSSSTCKSLA--IKDANVTGGFYG
.T.Q.....N.....A..NV.....V--..EN.K.....
.TA.D.TS.A.T..AM.KD--..FS.V.K.---GEN.FAL.PQN..N.HYTH.-E.T.S.....
.TAEN..AQT.T.E.M.Q.--..FE...K.---AES.FDL.QKN.TRTPKAY.T..K.K.....
.TAEN.SEAT.T.DAM.E.--..FK...K.---GND.FAP.QNNSIVTHKVH.AN.E.Q.....
.KRHDTCN.....EAFNNSS.AF....TA,-----NFV..GKNSQKNTPINITIK.N.A....

FIG.13F

680
FNANEMGGSFT-----NADDSKASV
.....-HDT.....
K..I.....SFGNAPECKQE-----
.K.E.L..W.AYPGDKQTEKATATSSDG---.SAS.-.T.
...E.L..W.AYPGNEQTKNATVESGNG---.SAS.-.T.
.K.S.L..Y..YNGNSTATNSESSTIVSSSS.SKNAP.A.

4223
Q8
B16B6
M982
FA19
Eagan

700
VFGTKRQQEV-K*
.....E.-.*
...A....L.Q-*
...A....P.Q-*
...A....KL.-.*
...ARQ.V.TT.*

4223
Q8
B16B6
M982
FA19
Eagan

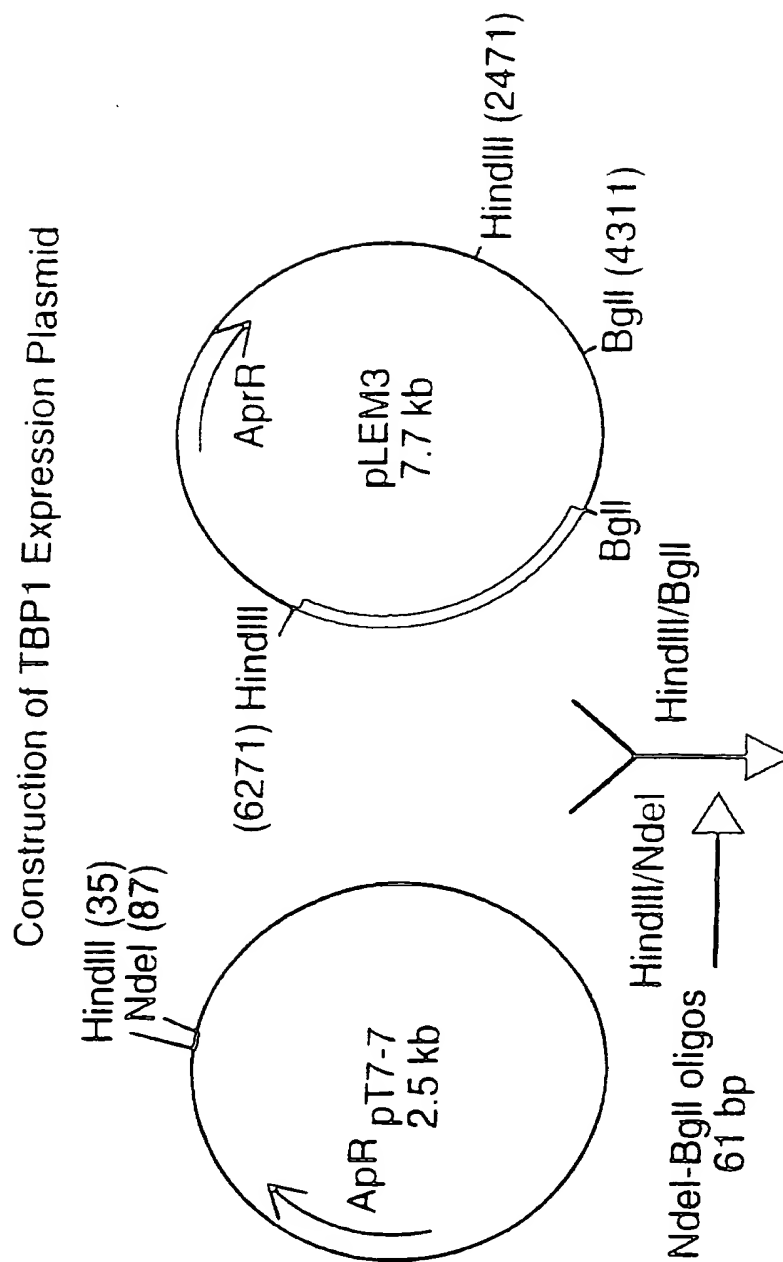


FIG. 14A

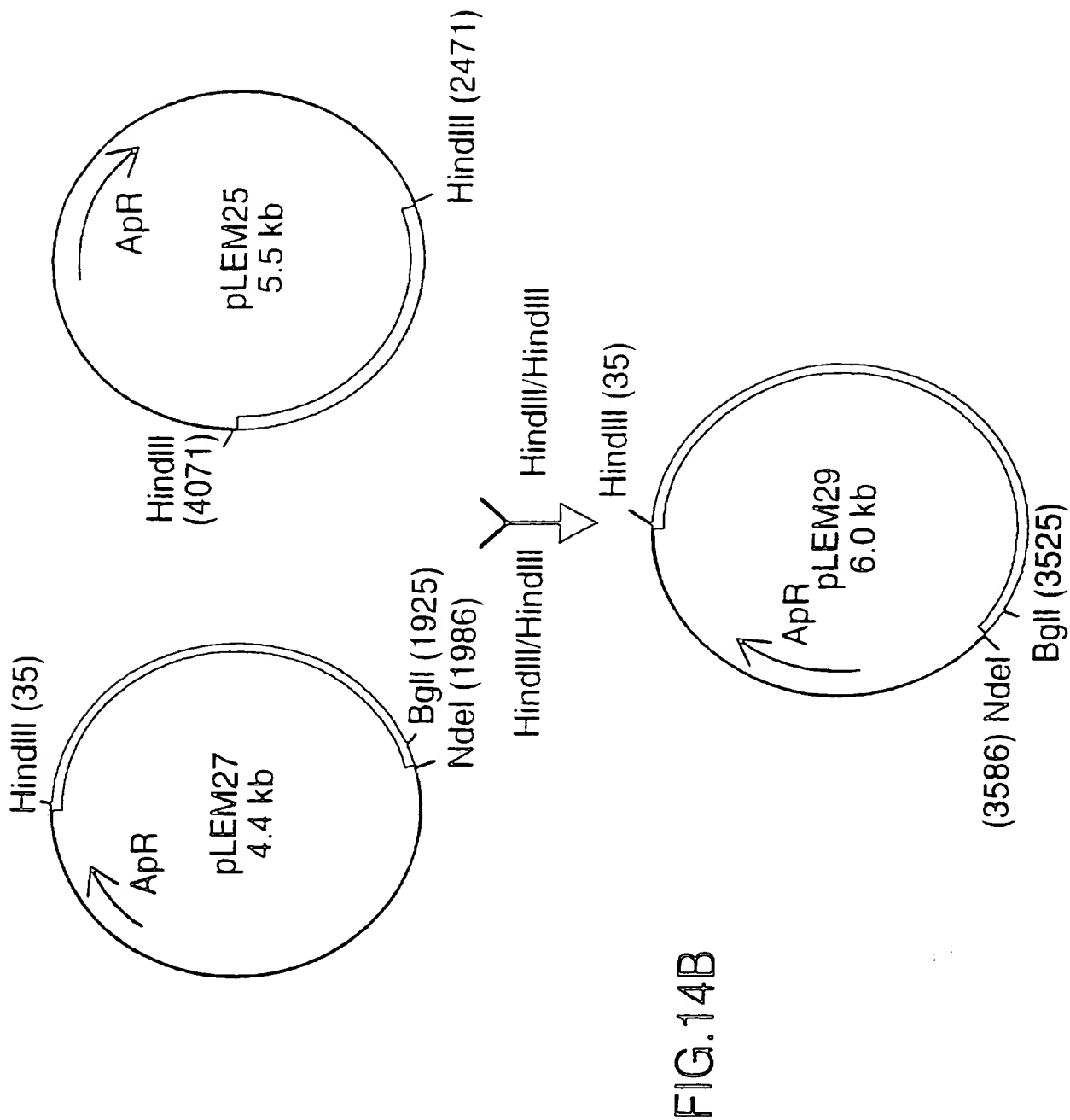
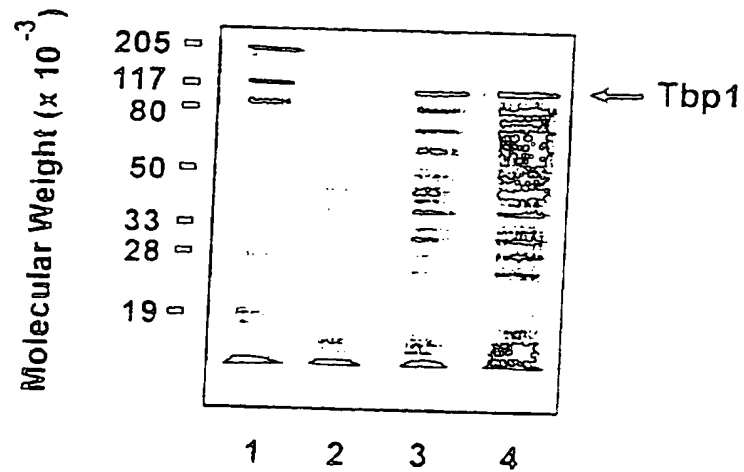


FIG.14B

Expression of rTbp1 in *E. coli*



1. Prestained molecular weight markers
2. pLEM29B-1 lysate, non-induced
3. pLEM29B-1 lysate, 1 hr post-induction
4. pLEM29B-1 lysate, 3 hr post-induction

Fig.15

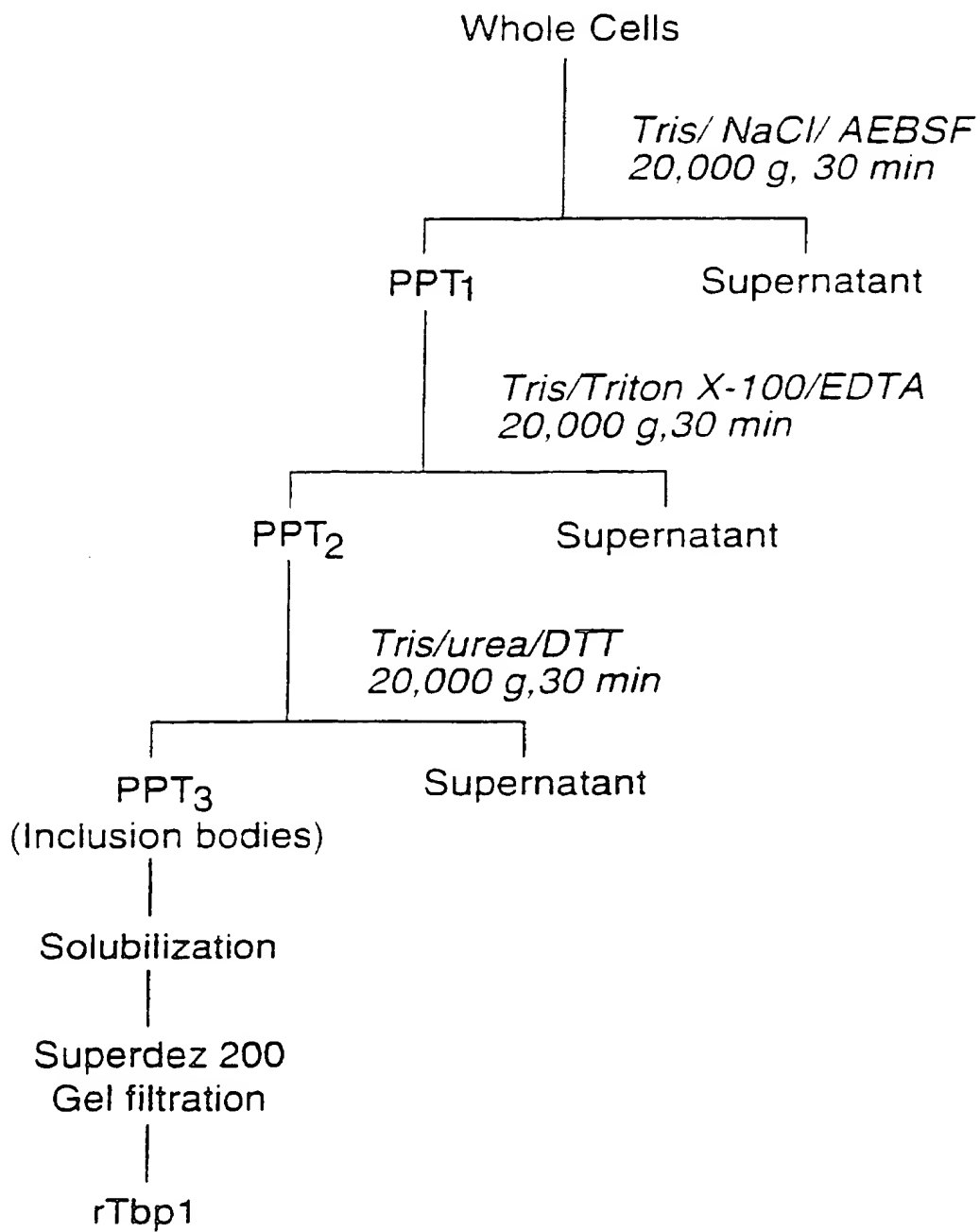
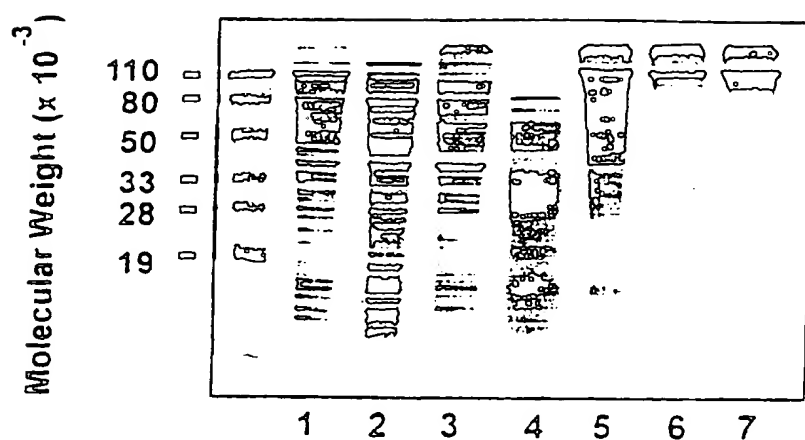
Purification of Tbp1 from *E. Cole*

FIG.16

Purification of rTbp1 from *E. coli*



1. *E. coli* Whole cells
2. Soluble proteins after 50 mM Tris/ NaCl extraction
3. Soluble proteins after Tris/ Triton X-100/ EDTA extraction
4. Soluble proteins after Tris/ urea/ DTT extraction
5. Left-over pellet (rTbp1 inclusion bodies)
- 6.7. Purified rTbp1

Fig.17

CONSTRUCTION OF TBP2 EXPRESSION PLASMID

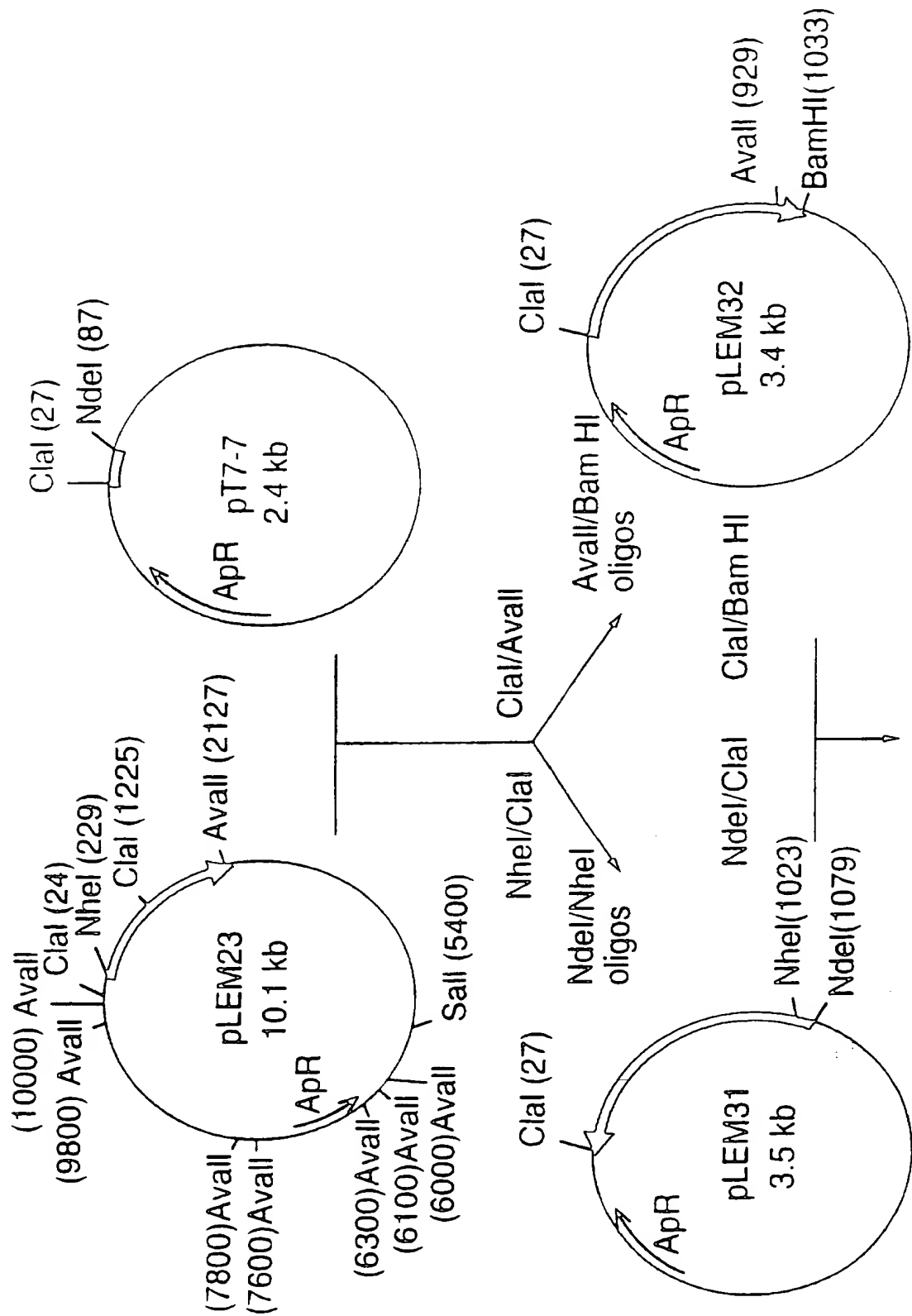


FIG.18A

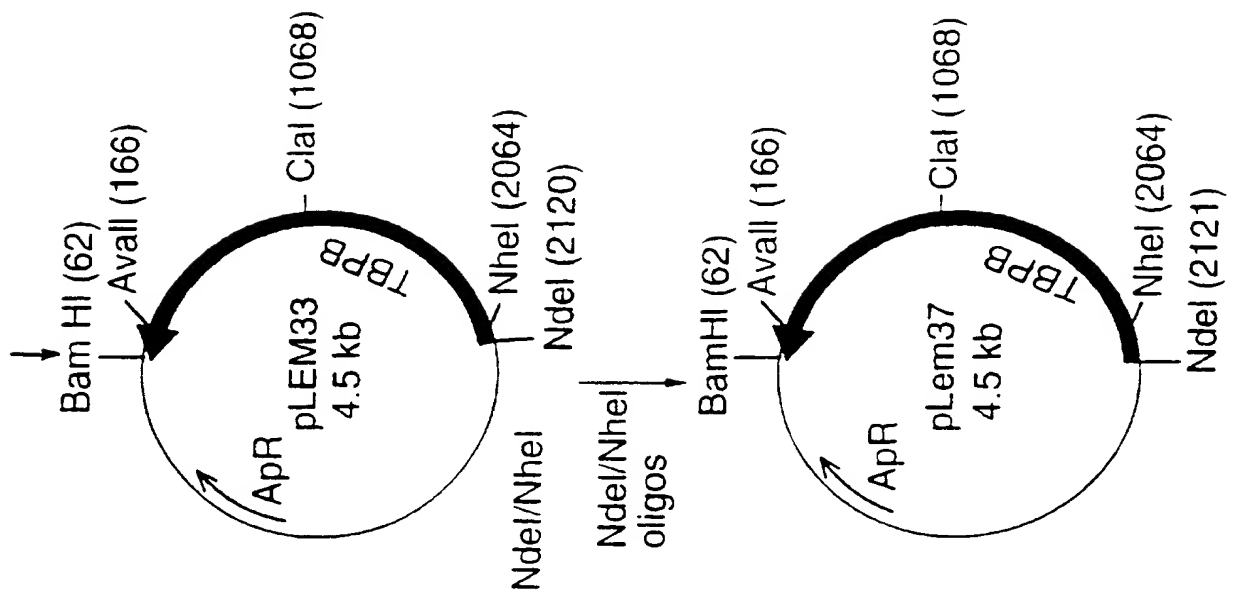
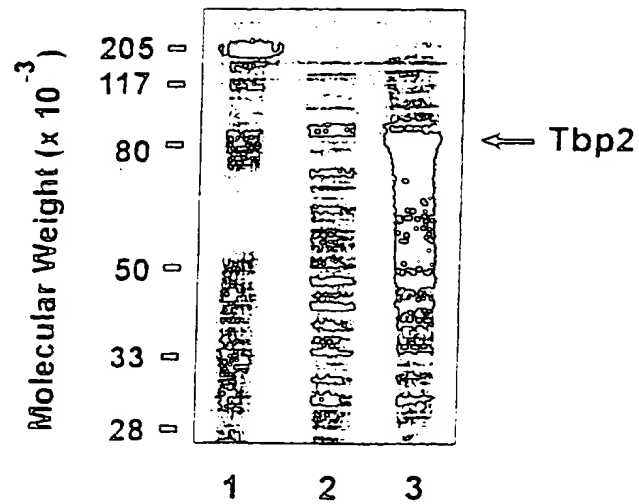


FIG.18B

SUBSTITUTE SHEET (RULE 26)

Expression of rTbp2 in *E. coli*



1. Prestained molecular weight markers
2. pLEM37B-2 lysate, non-induced
3. pLEM37B-2 lysate, induced

Fig.19

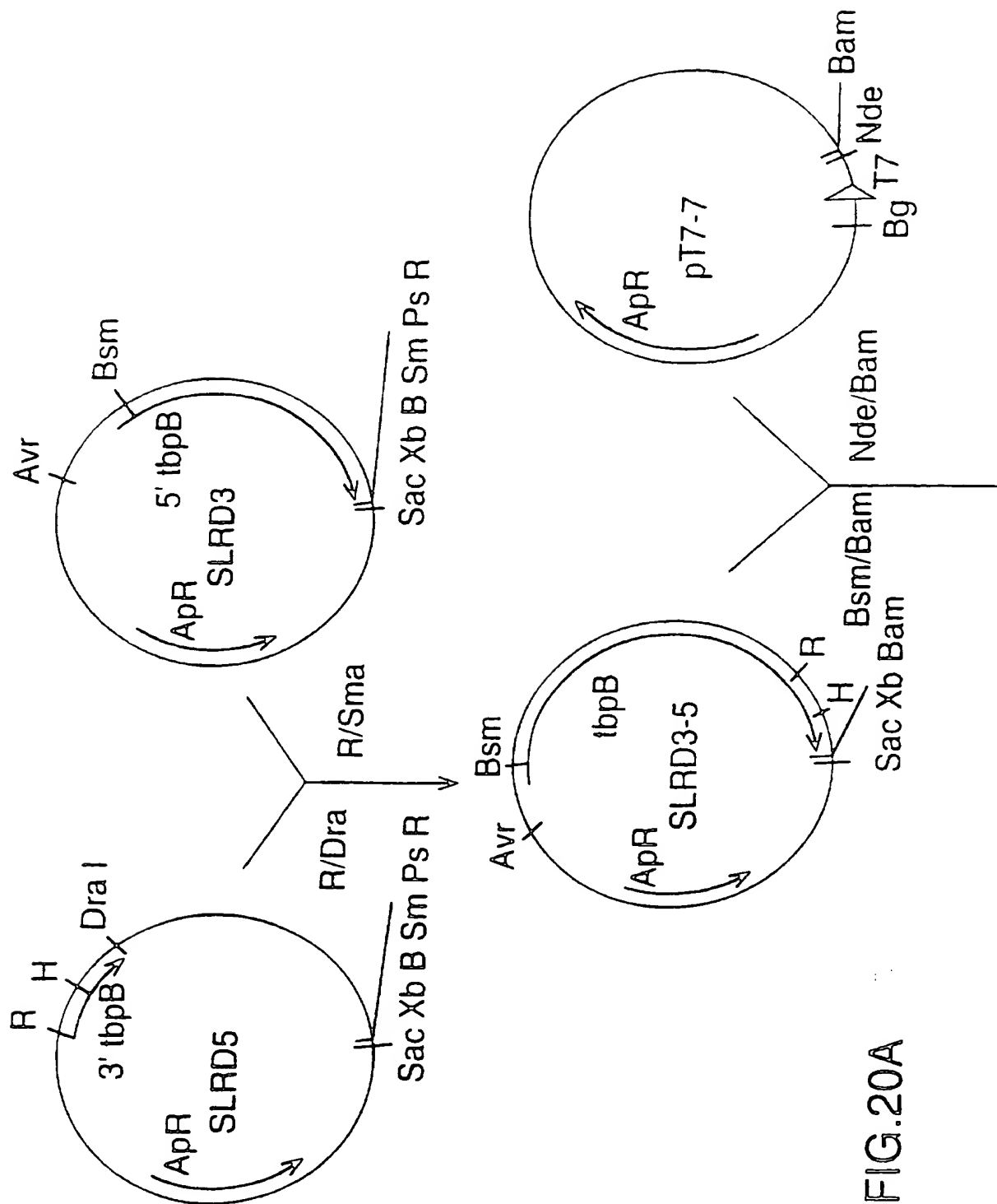


FIG.20A

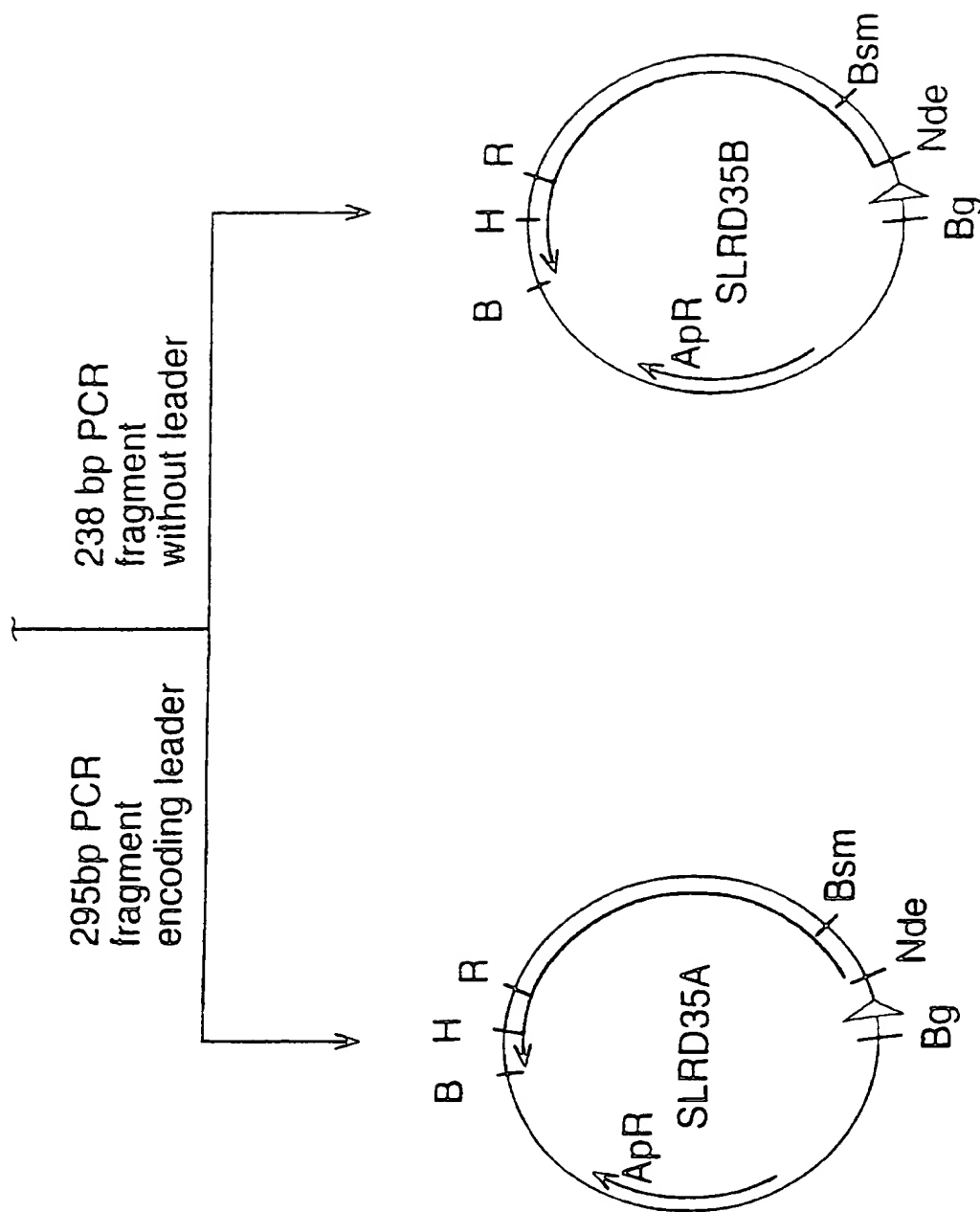
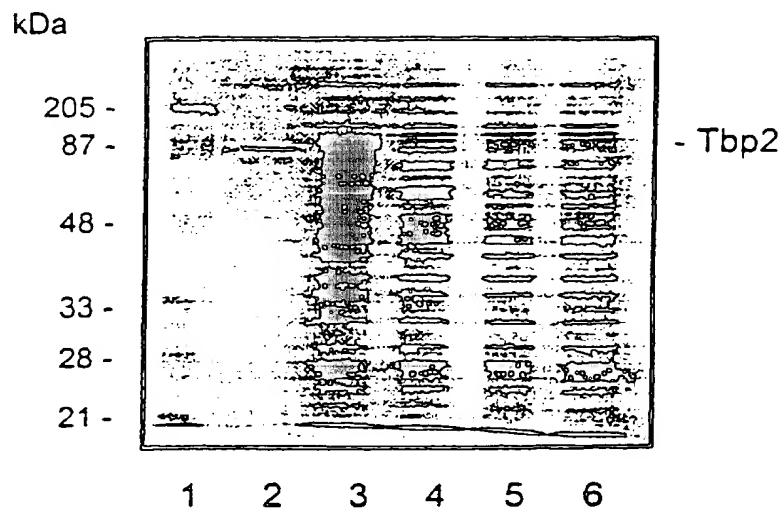


FIG.20B

Fig 21. Expression of Q8 rTbp2 protein in *E. coli*



1. Prestained molecular weight markers
2. 4223 rTbp2 protein
3. SLRD35A lysate, 3 hr post-induction
4. SLRD35B lysate, 3 hr post-induction
5. SLRD35A lysate, non-induced
6. SLRD35B lysate, non-induced

SUBSTITUTE SHEET (RULE 26)

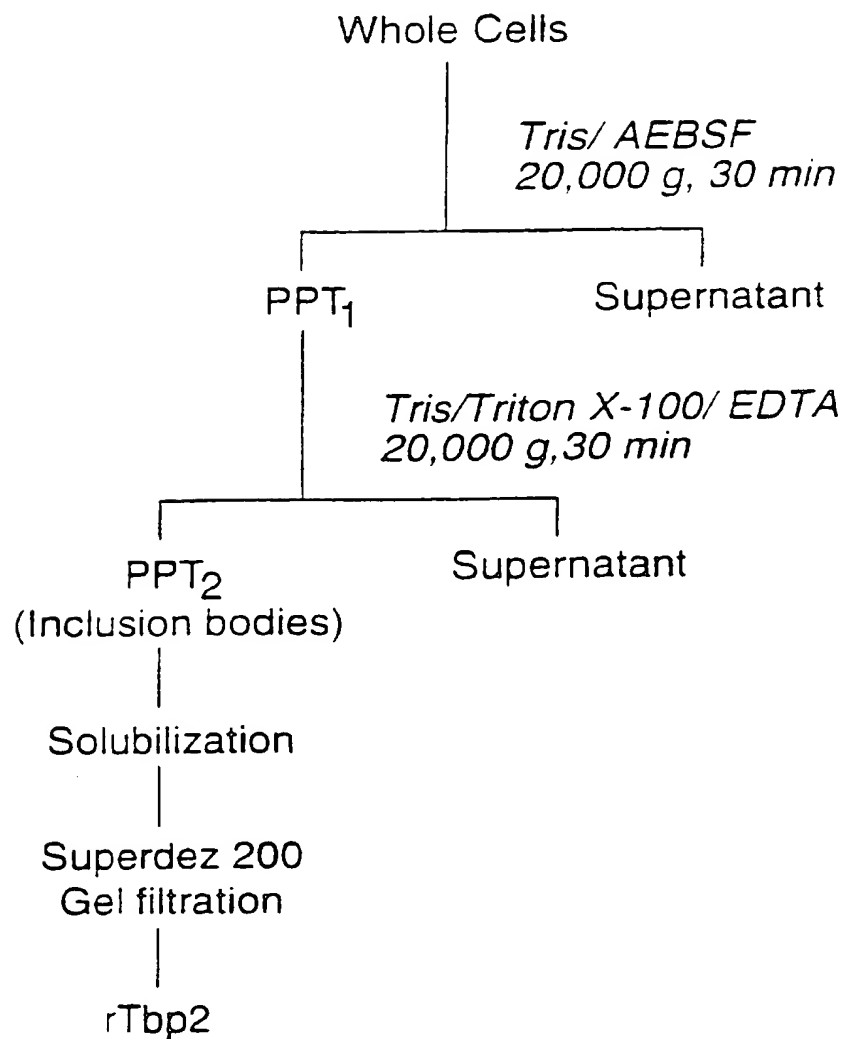
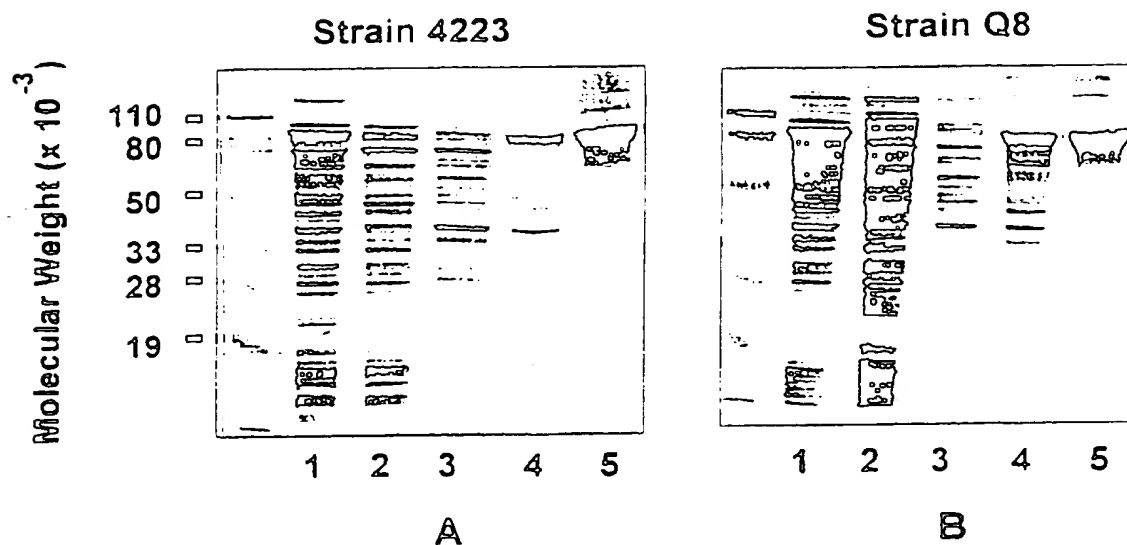
Purification of Tbp2 from *E.Coli*

FIG.22

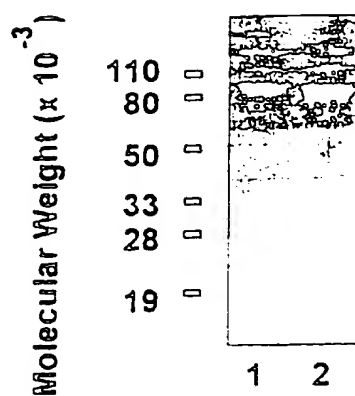
Purification of rTbp2 from *E. coli*



1. *E. coli* Whole cells
2. Soluble proteins after 50 mM Tris extraction
3. Soluble proteins after Tris/ Triton X-100/ EDTA extraction
4. Left-over pellet (rTbp2 inclusion bodies)
5. Purified rTbp2

Fig.23

Binding of Tbp2 to Human Transferrin



1. rTbp2 (strain 4223)

2. rTbp2 (strain Q8)

Fig.24

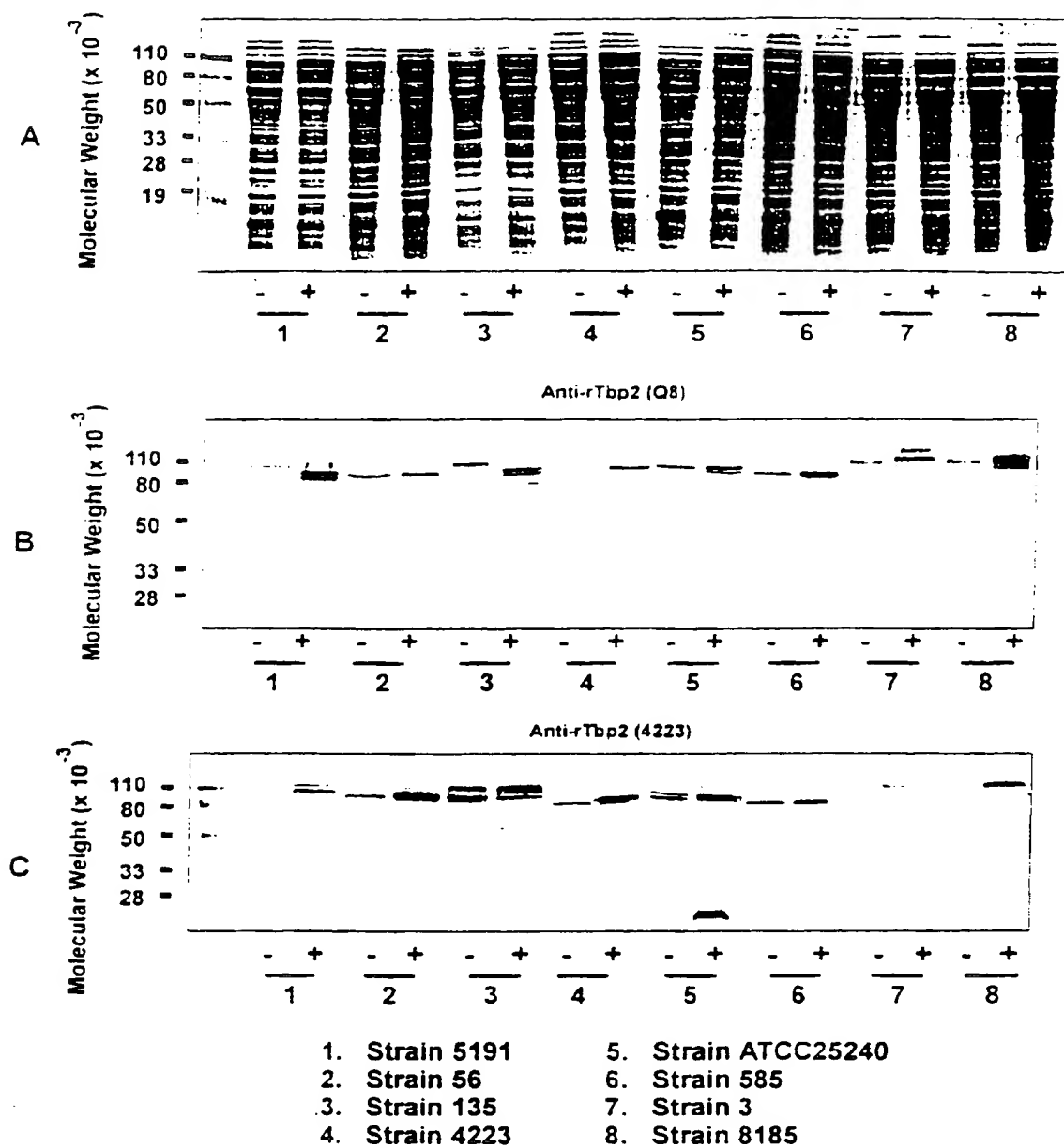


Fig.25

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Figure 27 Nucleotide and deduced amino acid sequence of *M. catenalis* R1 *tbpB*

AAATTTGCCGTATTTTGTCTATCATAAATGCATTATCATCAATGCCCAAAACAATACGCCAAATGCACAT
 TGTGAGCATGCCAAAATAGGCATTAAACAGACTTTTTTAGATAATACCATCAACCCATCAGAGGATTATTTT
 27 54
 ATG AAA CAC ATT CCT TTA ACC ACA CTG TGT GTG GCA ATC TCT GGC GTC TTA TTA
 MET Lys His Ile Pro Leu Thr Thr Leu Cys Val Ala Ile Ser Ala Val Leu Leu
 81 108
 ACC GCT TGT GGT GGC AGT GGT GGT TCA AAT CCA CCT GCT CCT ACG CCC ATT CCA
 Thr Ala Cys Gly Gly Ser Gly Gly Ser Asn Pro Pro Ala Pro Thr Pro Ile Pro
 135 162
 AAT GCT AGC GGT TCA GGT AAT ACT GGC AAC ACT GGT AAT GCT GGC GGT ACT GAT
 Asn Ala Ser Gly Ser Gly Asn Thr Gly Asn Thr Gly Asn Ala Gly Gly Thr Asp
 189 216
 AAT ACA GCC AAT GCA GGT AAT ACA GGC GGT ACA AGC TCT GGT ACA GGC AGT GCC
 Asn Thr Ala Asn Ala Gly Asn Thr Gly Gly Thr Ser Ser Gly Thr Gly Ser Ala
 243 270
 AGC ACG TCA GAA CCA AAA TAT CAA GAT GTG CCA ACA ACG CCC AAT AAC AAA GAA
 Ser Thr Ser Glu Pro Lys Tyr Gln Asp Val Pro Thr Thr Pro Asn Asn Lys Glu
 297 324
 CAA GTT TCA TCC ATT CAA GAA CCT GCC ATG GGT TAT GGC ATG GCT TTG AGT AAA
 Gln Val Ser Ser Ile Gln Glu Pro Ala MET Gly Tyr Gly MET Ala Leu Ser Lys
 351 378
 ATT AAT CTA TAC GAC CAA CAA GAC ACG CCA TTA GAT GCA AAA AAT ATC ATT ACC
 Ile Asn Leu Tyr Asp Gln Gln Asp Thr Pro Leu Asp Ala Lys Asn Ile Ile Thr
 405 432
 TTA GAC GGT AAA AAA CAA GTT GCT GAC AAT CAA AAA TCA CCA TTG CCA TTT TCG
 Leu Asp Gly Lys Lys Gln Val Ala Asp Asn Gln Lys Ser Pro Leu Pro Phe Ser
 459 486
 TTA GAT GTA GAA AAT AAA TTG CTT GAT GGC TAT ATA GCA AAA ATG AAT GAA GCG
 Leu Asp Val Glu Asn Lys Leu Leu Asp Gly Tyr Ile Ala Lys MET Asn Glu Ala
 513 540
 GAT AAA AAT GCC ATT GGT GAA AGA ATT AAG AGA GAA AAT GAA CAA AAT AAA AAA
 Asp Lys Asn Ala Ile Gly Glu Arg Ile Lys Arg Glu Asn Glu Gln Asn Lys Lys
 567 594
 ATA TCC GAT GAA GAA CTT GCC AAA AAA ATC AAA GAA AAT GTG CGT AAA AGC CCT
 Ile Ser Asp Glu Glu Leu Ala Lys Lys Ile Lys Glu Asn Val Arg Lys Ser Pro
 621 648
 GAG TTT CAG CAA GTA TTA TCA TCG ATA AAA GCG AAA ACT TTC CAT TCA AAT GAC
 Glu Phe Gln Gln Val Leu Ser Ser Ile Lys Ala Lys Thr Phe His Ser Asn Asp

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Fig 27 (cont.)

675 702
 AAA ACA ACC AAA GCA ACC ACA CGA GAT TTA AAA TAT GTT GAT TAT GGT TAC TAC
 Lys Thr Thr Lys Ala Thr Thr Arg Asp Leu Lys Tyr Val Asp Tyr Gly Tyr Tyr

729 756
 TTG GTG AAT GAT GGC AAT TAT CTA ACC GTC AAA ACA GAC AAC CCA AAA CTT TGG
 Leu Val Asn Asp Ala Asn Tyr Leu Thr Val Lys Thr Asp Asn Pro Lys Leu Trp

783 810
 AAT TCA GGT CCT GTG GGC GGT GTG TTT TAT AAT GGC TCA ACG ACC GGC AAA GAG
 Asn Ser Gly Pro Val Gly Gly Val Phe Tyr Asn Gly Ser Thr Thr Ala Lys Glu

837 864
 CTG CCC ACA CAA GAT GCG GTC AAA TAT AAA GGA CAT TGG GAC TTT ATG ACC GAT
 Leu Pro Thr Gln Asp Ala Val Lys Tyr Lys Gly His Trp Asp Phe MET Thr Asp

891 918
 GTT GCC AAA AAA AGA AAC CGA TTT AGC GAA GTA AAA GAA ACC TAT CAA GCA GGC
 Val Ala Lys Lys Arg Asn Arg Phe Ser Glu Val Lys Glu Thr Tyr Gln Ala Gly

945 972
 TGG TGG TAT GGG GCA TCT TCA AAA GAT GAA TAC AAC CGC TTA TTA ACC AAA GCA
 Trp Trp Tyr Gly Ala Ser Ser Lys Asp Glu Tyr Asn Arg Leu Leu Thr Lys Ala

999 1026
 GAT GCC GCA CCT GAT AAT TAT AGC GGT GAA TAT GGT CAT AGC AGT GAA TTT ACT
 Asp Ala Ala Pro Asp Asn Tyr Ser Gly Glu Tyr Gly His Ser Ser Glu Phe Thr

1053 1080
 GTT AAT TTT AAG GAA AAA AAA TTA ACA GGT GAG CTG TTT AGT AAC CTA CAA GAC
 Val Asn Phe Lys Glu Lys Lys Leu Thr Gly Glu Leu Phe Ser Asn Leu Gln Asp

1107 1134
 AGC CAT AAA CAA AAA GTA ACC AAA ACA AAA CGC TAT GAT ATT AAG GCT GAT ATC
 Ser His Lys Gln Lys Val Thr Lys Thr Lys Arg Tyr Asp Ile Lys Ala Asp Ile

1161 1188
 CAC GGC AAC CGC TTC CGT GGC AGT GCC ACC GCA AGC GAT AAG GCA GAA GAC AGC
 His Gly Asn Arg Phe Arg Gly Ser Ala Thr Ala Ser Asp Lys Ala Glu Asp Ser

1215 1242
 AAA AGC AAA CAC CCC TTT ACC ACC GAT GCC AAA GAT AAG CTA GAA GGT GGT TTT
 Lys Ser Lys His Pro Phe Thr Ser Asp Ala Lys Asp Lys Leu Glu Gly Gly Phe

1269 1296
 TAT GGA CCA AAA GGC GAG GAG CTG GCA GGT AAA TTC TTA ACC GAT GAT AAC AAA
 Tyr Gly Pro Lys Gly Glu Glu Leu Ala Gly Lys Phe Leu Thr Asp Asp Asn Lys

1323 1350
 CTC TTT GGT GTC TTT GGT GCC AAA CAA GAG GGT AAT GTA GAA AAA ACC GAA GCC
 Leu Phe Gly Val Phe Gly Ala Lys Gln Glu Gly Asn Val Glu Lys Thr Glu Ala

Fig 27 (cont)

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1377 1404
 ATC TTA GAT GGT TAT GCA CTT GGS ACA TTT AAT AAA CCT GGT ACC ACC AAT CCC
 Ile Leu Asp Ala Tyr Ala Leu Gly Thr Phe Asn Lys Pro Gly Thr Thr Asn Pro

1431 1458
 GCC TTT ACC GGT AAC AGC AAA AAA GAA CTG GAT AAC TTT GGC AAT GCC AAA AAG
 Ala Phe Thr Ala Asn Ser Lys Lys Glu Leu Asp Asn Phe Gly Asn Ala Lys Lys

1485 1512
 TTG GTC TTG GGT TCT ACC GTC ATT GAT TTG GTG CCT ACT GAT GGC ACC AAA GAT
 Leu Val Leu Gly Ser Thr Val Ile Asp Leu Val Pro Thr Asp Ala Thr Lys Asp

1539 1566
 GTC AAT GAA TTC AAA GAA AAG CCA AAG TCT GCC ACA AAC AAA GCG GGC GAA ACT
 Val Asn Glu Phe Lys Glu Lys Pro Lys Ser Ala Thr Asn Lys Ala Gly Glu Thr

1593 1620
 TTG ATG GTG AAT GAT GAA GTT AGC GTC AAA ACC TAT GGC AAA AAC TTT GAA TAC
 Leu MET Val Asn Asp Glu Val Ser Val Lys Thr Tyr Gly Lys Asn Phe Glu Tyr

1647 1674
 CTA AAA TTT GGT GAG CTT AGT GTC GGT GGT AGC CAT AGC GTC TTT TTA CAA GGC
 Leu Lys Phe Gly Glu Leu Ser Val Gly Gly Ser His Ser Val Phe Leu Gln Gly

1701 1728
 GAA GGC ACC GGT ACC ACA GGC GAG AAA GCC GTA CCA ACC ACA GGC AAA GCC AAA
 Glu Arg Thr Ala Thr Thr Gly Glu Lys Ala Val Pro Thr Thr Gly Lys Ala Lys

1755 1782
 TAT TTG GGG AAC TGG GTA GGA TAT ATC ACA GGA GCG GAC TCA TCA AAA GGC TCT
 Tyr Leu Gly Asn Trp Val Gly Tyr Ile Thr Gly Ala Asp Ser Ser Lys Gly Ser

1809 1836
 ACC GAT GGC AAA GGC TTT ACC GAT GCC AAA GAT ATT GCT GAT TTT GAC ATT GAC
 Thr Asp Gly Lys Gly Phe Thr Asp Ala Lys Asp Ile Ala Asp Phe Asp Ile Asp

1863 1890
 TTT GAG AAA AAA TCA GTT AAT GGC AAA CTG ACC ACC AAA GAC CGC CAA GAC CCT
 Phe Glu Lys Lys Ser Val Asn Gly Lys Leu Thr Thr Lys Asp Arg Gln Asp Pro

1917 1944
 GTC TTT AAC ATC ACA GGT GAA ATC GCA GGC AAT GGC TGG ACA GGT AAA GCC AGC
 Val Phe Asn Ile Thr Gly Glu Ile Ala Gly Asn Gly Trp Thr Gly Lys Ala Ser

1971 1998
 ACC GCG GAA GCG AAC GCA GGG GGC TAT AAG ATA GAT TCT AGC AGT ACA GGC AAA
 Thr Ala Glu Ala Asn Ala Gly Gly Tyr Lys Ile Asp Ser Ser Ser Thr Gly Lys

2025 2052
 TCC ATC GTC ATC AAA GAT GCC GTG GTT ACA GGT GGT TTT TAT GGT CCA AAT GCA
 Ser Ile Val Ile Lys Asp Ala Val Val Thr Gly Gly Phe Tyr Gly Pro Asn Ala

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Fig 27 (cont)

2079

2106

ACC GAG ATG GGT GGG TCA TTT ACA CAC AAC AGC GGT AAT GAT GGT AAA CTC TCT
Thr Glu MET Gly Gly Ser Phe Thr His Asn Ser Gly Asn Asp Gly Lys Val Ser

2133

GTG GTC TTT GGC ACA AAA AAA CAA GAA GTT AAG AAG TGA
Val Val Phe Gly Thr Lys Lys Gln Glu Val Lys Lys *

Alignment of *M. catarrhalis* Tbp2

BNSDOCID: <WO 9732980A1>

INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/CA 97/00163

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/22 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 97 13785 A (CONNAUGHT LAB ; YANG YAN PING (CA); MYERS LISA E (CA); HARKNESS ROB) 17 April 1997 see the whole document	1-25
Y	WO 90 12591 A (UNIV TECHNOLOGIES INTERNATIONA ; SCHRYVERS ANTHONY BERNARD (CA)) 1 November 1990 see claims 1-26 --- -/--	1-25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *@* document member of the same patent family

Date of the actual completion of the international search

17 July 1997

Date of mailing of the international search report

30 JULY 1997 (30.07.97)

Name and mailing address of the ISA

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Fax: (+ 31-70) 340-3016

Authorized officer

Nauche, S

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/CA 97/00163

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MICROBIAL PATHOGENESIS, vol. 15, 1993, pages 433-445, XP000612196 RAONG-HUA YU ET AL: "THE INTERACTION BETWEEN HUMAN TRANSFERRIN AND TRANSFERRIN BINDING PROTEIN 2 FROM MORAXELLA (BRANHAMELLA) CATARRHALIS DIFFERS FROM THAT OF OTHER HUMAN PATHOGENS" see the whole document ---	1-25
A	WO 95 33049 A (PASTEUR MERIEUX SERUMS VACC ;TRANSGENE SA (FR); MILLET MARIE JOSE) 7 December 1995 see the whole document ---	1-25
A	WO 93 08283 A (UNIV SASKATCHEWAN) 29 April 1993 see the whole document -----	1-25

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

page 2 of 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 97/00163

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 23
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 23 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 97/00163

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9713785 A	17-04-97	AU 7208296 A	30-04-97
WO 9012591 A	01-11-90	US 5292869 A	08-03-94
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		NZ 247967 A	24-02-95
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		AU 2675795 A	21-12-95
		CA 2167936 A	07-12-95
		EP 0720653 A	10-07-96
		FI 960428 A	28-03-96
		HU 75992 A	28-05-97
		JP 9501059 T	04-02-97
		NO 960332 A	21-03-96
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		AU 2751392 A	21-05-93
		CA 2121364 A	29-04-93
		EP 0610260 A	17-08-94
		US 5521072 A	28-05-96